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Feature Article

Challenges and Future Directions of Predatory Mites as Biological Control Agents under Climate Change

Jhih-Rong Liao[1,*], Chyi-Chen Ho[2,3], and Chiun-Cheng Ko[4,†]

Abstract

Liao, J. R., C. C. Ho, and C. C. Ko. 2024. Challenges and future directions of predatory mites as biological control agents under climate change. J. Taiwan Agric. Res. 73(3):153−168.

Climate change significantly challenges agricultural ecosystems, impacting pest and natural enemy dynamics and the effectiveness of biological control. Predatory mites (phytoseiid mites), crucial in the sustainable management of various agricultural pests, are particularly susceptible to these changing conditions. This review focuses on the influence of climate change on the biological control potential of predatory mites, identifying critical gaps in current knowledge that limit the development of climate-resilient biological control strategies. It examines how altered temperature regimes, precipitation patterns, and extreme weather events affect the physiology, distribution, and interactions of predatory mites with their prey. The review highlights the necessity for adaptive strategies to preserve and enhance the biological control efficacy of predatory mites against climate change. Through case studies, we illustrate practical implications and adaptive measures in managing predatory mites under changing climatic conditions. Urging dedicated research into species-specific climatic adaptability and the enhancement of predictive modeling for biological control outcomes, this analysis emphasizes the imperative for innovative management practices to tackle the challenges posed by climate change. A holistic approach, merging ecological, genetic, and technological insights, is crucial to sustain the functionality of biological control systems in a warming world.

Key words: Climate resilience, Pest management, Adaptation strategies, Ecosystem dynamics, Sustainable agriculture.

INTRODUCTION

The quest for sustainable pest management strategies has emphasized the critical role of natural enemies within agricultural ecosystems, with predatory mites from the Phytoseiidae family at the forefront. Controlling a broad spectrum of agricultural pests such as spider mites, thrips, and whiteflies have been a key focus of global research and application since the 1970s. This shift towards biological control methods was driven by concerns over the environmental and health impacts of chemical pesticides (Huffaker *et al*. 1970; McMurtry *et al*. 1970, 2013, 2015; Chant & McMurtry 2007). Phytoseiid mites are known for their remarkable adaptability and high rates of growth and reproduction under favorable conditions, transitioning rapidly through 4 immature stages and with mated females capable of producing several eggs per

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[†] Deceased, 29 October 2020. This paper is dedicated to the memory of the late Chiun-Cheng Ko.

day (McMurtry *et al*. 2013; Ghazy *et al*. 2018; Zhang *et al*. 2019). This biological potential facilitates their commercial mass production for biological control applications. Yet, in natural environments, large populations of phytoseiid mites are uncommon, suggesting that a complex interplay of environmental factors influences their abundance (Ghazy *et al*. 2016).

Globally, more than 2,700 phytoseiid species have been recorded, demonstrating significant biodiversity that is invaluable in combating a variety of agricultural pests (Demite *et al*. n.d.; Chant & McMurtry 2007). Severty phytoseiid species, including novel discoveries, and introduced species have been recorded in Taiwan, underscoring the country's critical role in phytoseiid research and global biodiversity contributions (Liao *et al*. 2020a, 2021b, 2023a, 2023b). Despite its small size, Taiwan's contribution is notable compared to the United States with 318 species and Japan with 101 species (Demite *et al*. n.d.). This underscores the need for further exploration of phytoseiid biodiversity, potentially revealing species with untapped biological control potential or enhanced climate resilience.

Climate change significantly impacts agricultural ecosystems by creating complex interactions among plants, herbivorous pests, natural enemies, and the environment. These tritrophic interactions become even more intricate when additional trophic levels such as pollinators and decomposers are considered. This leads to multifaceted effects, including direct impacts on species' physiology and behavior, as well as indirect effects through altered species interactions and ecosystem processes (Tylianakis *et al*. 2008; Thomson *et al*. 2010; Gerard *et al*. 2013; Welch & Harwood 2014; Han *et al*. 2019; Harvey *et al*. 2023). Temperature fluctuation, a key aspect of global climate change (Naz *et al*. 2022), poses significant challenges to the balance between phytoseiid mites and their prey (Ghazy *et al*. 2016). This creates a complex scenario, which both direct and indirect effects of climate change on natural enemies and their prey remain largely unpredictable (Fig. 1) (Nechols 2021). The behavioral and physiological parameters of these mites, central to their survival, development, and reproduction, are significantly influenced by the ambient temperature. Extreme temperatures can provoke stress, reducing survival and reproductive efficiency, or even leading to death under chronic conditions (Salt 1961; Denlinger 1991; Morewood 1992; Veerman 1992; Broufas & Koveos 2001; Ghazy *et al*. 2016; Zhang *et al*. 2016; Nakai *et al*. 2021). In addition, heat waves lead to differential responses between natural enemies and prey, with implications for biological control outcomes (Tscholl *et al*. 2023). Despite these challenges, phytoseiid mites exhibit remarkable adaptability, having evolved strategies like hibernation and reproductive diapause to survive harsh conditions. These adaptation measures underscore the need for in-depth research into species-specific responses to temperature extremes, which are becoming more unpredictable with climate change. This emphasizes the importance of strategic adaptation to maintain the efficacy of phytoseiid mites as biocontrol agents in the face of warming climates (Nechols 2021).

This article reviews the current researches on how climate change impacts the biological control potential of predatory mites and explores adaptation strategies to sustain their effectiveness. By identifying key research gaps and suggesting future directions, this review aims to enhance biological control practices amidst climatic uncertainties, supporting sustainable agricultural ecosystems through better understanding and adaptation to environmental stresses affecting phytoseiid mites.

PHYTOSEIID MITES: PILLARS OF BIOLOGICAL CONTROL

Phytoseiid mites, belonging to the family Phytoseiidae, are paramount in the arsenal of biological control agents used in agricultural ecosystems. With over 2,700 species across 90 genera globally, these mites demonstrate signifi-

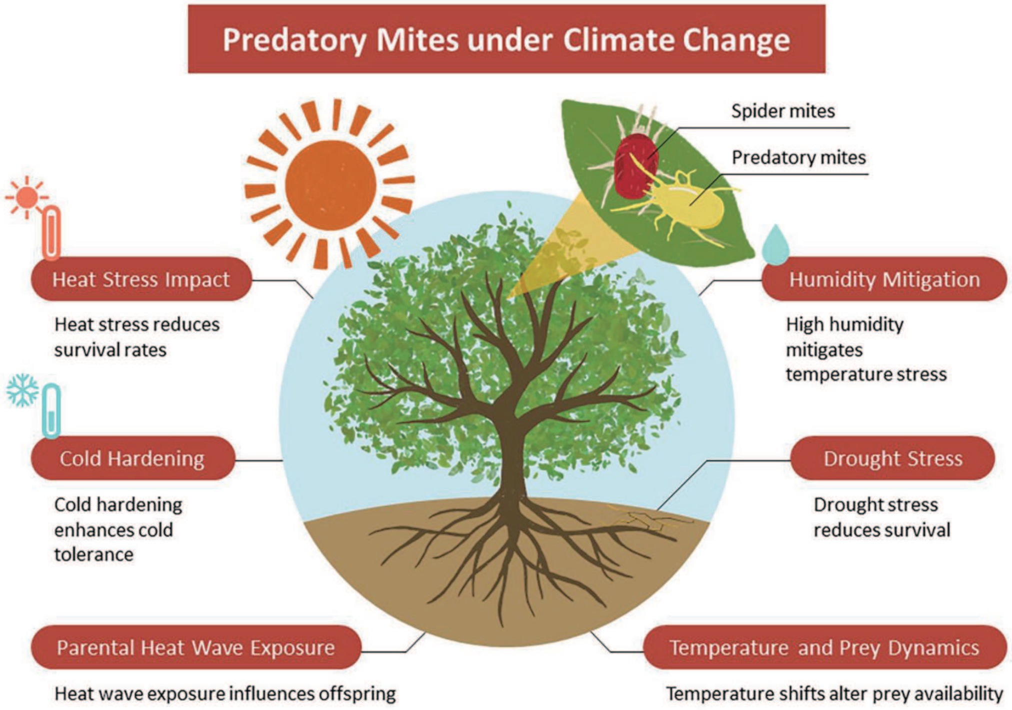

Fig. 1. Climate change affects predatory mites as biological control agents through multiple trophic levels, impacting their interactions with prey, host plants, and the environment.

cant diversity and adaptability—traits that make them formidable adversaries against a range of agricultural pests (Demite *et al.* n.d.; Chant & McMurtry 2007). The lifestyles are classified into 4 major types based on feeding habits, from specialized mite predators to pollen-feeding generalists, allowing for tailored biological control strategies to match specific pest scenarios (McMurtry & Croft 1997; McMurtry *et al.* 2013). Type I mites are efficient predators of *Tetranychus* spider mites, suitable for controlled agricultural environments where these pests are prevalent. Type II species are selective predators of tetranychid mites. Type III species are generalist predators that engage in a wide range of prey, while Type IV mites thrive in environments where pollen is readily available, demonstrating their utility as generalist predators in diversified crop systems. Subsequently, McMurtry *et al.* (2013) classified the major 4 lifestyles into more several substyles based on the diverse habitats, from plants to soils. This diversity ensures that phytoseiids can be effectively employed against a wide range of agricultural pests, including spider mites, thrips, and whiteflies, which are common threats to crops worldwide (Huffaker *et al.* 1970; McMurtry *et al.* 1970, 2013, 2015). This adaptability extends to their physiological resilience against environmental stressors such as temperature and humidity, crucial under the evolving threat of climate change (Ghazy *et al.* 2016; Harvey *et al.* 2023).

Taiwan and its affiliated islands, known for their rich biodiversity, have been reported to have 70 species of phytoseiids. This underscores the region's importance in phytoseiid research and potential in biological control applications (Liao *et al.* 2020a, 2023b). Taiwan has been at the forefront of phytoseiid mite research, significantly contributing to the understanding and application of these mites in biological control. Early researches by P. K. C. Lo and C. I. T. Shih on *Neoseiulus longispinosus* (Evans), later correctly identified as *N. womer-*

sleyi (Schicha), laid the foundation for innovative mass-rearing techniques and practical field applications (Schicha 1975; Lo & Ho 1979; Shih & Shieh 1979; Ho 2005). Their pioneering work facilitates the control of spider mites in various orchards, including strawberry fields and tea plantations, showcasing the practical application and adaptability of phytoseiids in Taiwan's unique agricultural landscape (Lo *et al*. 1984, 1986; Ho 1990). Continued efforts by Taiwanese researchers have further demonstrated the adaptability and effectiveness of local predatory mite species, such as *N. womersleyi* and *Euseius ovalis* (Evans), in sustainable pest management strategies (Shih *et al*. 1993; Shih & Wang 1997; Ho & Chen 2001, 2002a, 2002b; Ho 2005). Moreover, commercial endeavors by local companies, such as Good Farms, have illustrated the successful transition from research to market, developing products based on *N. barkeri* Hughes (Liao *et al*. 2020a), which offer higher adaptability in the field and a broader range of prey.

The growing impacts of climate change—temperature fluctuations, altered precipitation patterns, heat waves, and increased frequency of extreme weather events—underscore the need for continued research into the ecological roles and adaptability of phytoseiids (Ghazy *et al*. 2016; Tscholl *et al*. 2023). Recent discoveries of phytoseiid biodiversity in Taiwan (Liao *et al*. 2020a) enhance the arsenal of biological control agents, offering new avenues for pest management in the face of climate shifts. Optimizing the use of phytoseiids in agriculture through understanding their responses to environmental stressors and developing innovative strategies to enhance their effectiveness is essential for the sustainability of agricultural ecosystems and food security (Welch & Harwood 2014).

CLIMATE CHANGE IMPACTS ON AGRICULTURAL ECOSYSTEMS AND PREDATORY MITES

Climate change induces profound environmental alterations, affecting every component of agricultural ecosystems, from water availability to the dynamics of pests and their natural enemies (Loboguerrero *et al*. 2019; Skendžić *et al*. 2021; Naz *et al*. 2022). Extreme weather events, such as prolonged droughts, severe flooding, heatwaves, and heavy storms, exacerbate these impacts, causing direct harm to crops and altering the habitats of pests. These climatic changes can unpredictably shift the presence and abundance of pests, potentially diminishing the effectiveness of biological control strategies (Gillespie *et al*. 2012; Ghazy *et al*. 2016; Zhang *et al*. 2016; Nakai *et al*. 2021; Nechols 2021; Tscholl *et al*. 2023).

For phytoseiid mites, temperature stands out as a pivotal abiotic factor deeply influencing their behavior and physiological parameters (Ghazy *et al*. 2016). Temperature, largely dictated by ambient conditions, directly and indirectly influences their survival, development, and reproduction (Veerman 1992; Ghazy *et al*. 2016; Han *et al*. 2019). Extreme temperatures, either too high or too low, can provoke significant stress, reducing their survival rates, interrupting development and reproduction processes, or even leading to death under chronic exposure (Tscholl *et al*. 2023). Adaptation measures such as hibernation and reproductive diapause have evolved in phytoseiid mites to withstand these harsh temperature conditions, showcasing their resilience and the critical need for strategic adaptation to sustain their biological control potential amidst climate change (Salt 1961; Denlinger 1991; Veerman 1992; Teets & Denlinger 2013; Ghazy *et al*. 2016). Rising temperatures may facilitate the escape and establishment of introduced predators from greenhouses into the surrounding environment (Hart *et al*. 2002). In addition, Tscholl *et al*. (2023) highlighted that parental exposure to heat waves can influence the reproductive investment of subsequent generations of predatory mites. This transgenerational effect suggests that the biological control efficacy of phytoseiid mites could be shaped by the climatic experiences of their

ancestors, adding a layer of complexity to predicting the performance of biological control agents under climate extremes.

Cold stress, manifesting through chilling or freezing, requires phytoseiid mites to employ mechanisms like cold hardening to enhance their cold tolerance—a genetic trait triggered by environmental stimuli such as decreasing temperature and shortening day length (Salt 1961; Denlinger 1991; Teets & Denlinger 2013). Conversely, high temperatures combined with limited access to prey can exacerbate drought stress, dehydrating mites, and potentially leading to their demise. Environments with high humidity may mitigate the severity of both temperature extremes and food shortages, underlining the complex interplay between temperature, humidity, and food availability in determining the biological control efficacy of phytoseiid mites under the rapidly changing climatic conditions (Morewood 1992; Broufas & Koveos 2001; Nakai *et al*. 2021). Amid these challenges, the adaptability of phytoseiid mites to changing climates, as demonstrated by classical biological control (CBC) of 3 exotic phytoseiids in Taiwan, offers valuable insights. These researches highlight the importance of ecological niche modeling in predicting biological control success under varied climatic conditions, emphasizing strategic species selection for future pest management scenarios (Liao *et al*. 2021a, 2023a).

As we advance, prioritizing research that anticipates the responses of both pest and predator populations to climate change is crucial. Designing effective, climate-resilient biological control programs hinges on our understanding of the adaptability and resilience of phytoseiid mites against climate change. This underscores the importance of continued innovation and adaptation in biological control strategies, ensuring the effectiveness of phytoseiid mites as biological control agents. To illustrate the practical implications of these challenges and adaptations, we discuss 2 case studies that highlight the impact of climate change on predatory mites. These examples demonstrate the complexities involved and offer insights into potential strategies for sustaining agricultural production and ecosystem health in a warming world.

CASE STUDY 1: CLASSICAL BIOLOGICAL CONTROL OF 3 EXOTIC PREDATORY MITES IN TAIWAN

Taiwan's innovative approach to agricultural pest management in the 1980s included the introduction of 3 exotic phytoseiid mite species: *Phytoseiulus persimilis* Athias-Henriot, *N. californicus* (McGregor) (Fig. 2), and *N. fallacis* (Garman). Lots of exotic predatory mites were released across diverse agricultural areas, from alpine orchards to tea plantations (Lo *et al*. 1986; Lee & Lo 1989; Ho 1990; Lo *et al*. 1990; Hao *et al*. 1996). This initiative aimed to leverage CBC program to combat spider mite infestations effectively. Initial post-release observations indicated an establishment only for *N. fallacis*, evidenced by its significant presence in the release fields (Lo *et al*. 1986). However, all 3 species were absent in subsequent long-term investigations (Liao *et al*. 2020a), suggesting a failure in its establishment. Liao *et al*. (2021a, 2023a) conducted comprehensive field investigations across Taiwan and applied ecological niche modeling to assess the establishment success of the 3 phytoseiid species. The results confirmed that only *N. californicus* successfully established in high mountain orchards (Fig. 2), while *P. persimilis* and *N. fallacis* failed to become sustainable parts of the agroecosystem. The modeling results matched the field observations, indicating that the unsuitable climatic conditions in Taiwan contributed to the unsuccessful establishment of *P. persimilis* and *N. fallacis*. This differential success among the introduced species illustrates the nuanced interaction between predatory mites and their new environments, emphasizing the critical role of selecting species with ecological compatibility for CBC programs. The contrasting outcomes

Fig. 2. *Neoseiulus californicus* established population on high mountain orchards. (A) Peach orchard in high mountain areas; and (B) adult female feeding on *Tetranychus urticae* on peach leaves.

of these introductions serve not only as a testament to the potential of CBC within specific ecosystems but also as a cautionary narrative about the complexities introduced by climate and environmental suitability. The success of *N. californicus*, in contrast to the failures of *N. fallacis* and *P. persimilis*, calls for a deeper exploration into how climatic conditions influence the establishment and efficacy of predatory mites as biological control agents.

Predictive models, particularly MaxEnt, have shown that current climatic conditions

favor *N. californicus* over *N. fallacis*, explaining their differential establishment outcomes. Yet, the shadow of climate change looms large, threatening to contract the suitable habitats for both species within Taiwan. This potential contraction signifies a pressing need for adaptive management strategies within CBC programs to accommodate the shifting environmental thresholds dictated by climate change. To navigate these challenges, a standardized assessment procedure becomes paramount. Cédola *et al.* (2021) proposed a framework essential for gauging the environmental fit and impact of introducing exotic predators. Advancements in molecular tools, such as metabarcoding, pave the way for precise identification of predatory mites, simplifying community-level assessments (Ollivier *et al.* 2020). Additionally, the integration of machine learning into CBC offers a novel avenue for enhancing species identification with unprecedented speed and accuracy (Liao *et al.* 2020b).

The narrative of *N. californicus* in Taiwan not only celebrates the triumphs of CBC but also serves as a cautionary tale about the complexities introduced by climate change. The case underscores an urgent call for research dedicated to understanding the nuanced effects of climatic shifts on biological control agents. Future investigations should prioritize species-specific assessments, particularly focusing on how altered temperature ranges, precipitation patterns, and the frequency of extreme weather events influence predatory mite populations and their prey dynamics. This comprehensive approach, marrying cutting-edge identification techniques with environmental risk assessments, is key to fortifying the resilience of CBC programs against climate change. By addressing the differential impacts of these environmental shifts, researchers and practitioners can better tailor biological control strategies, ensuring the sustainability of agricultural productivity and ecosystem health for generations to come.

CASE STUDY 2: ADAPTATION OF PREDATORY MITES TO CLIMATE CHANGE WITH POLLENS

Pollen has always played a crucial role in the research of phytoseiid mites due to its importance as a food source. McMurtry & Croft (1997) and McMurtry *et al.* (2013) classify certain phytoseiids as phytophagous or generalist type IV feeders, noting their use of pollen as an alternative food source. The potential of pollen as a substitute food for mass rearing natural enemies has been a subject of research (Shih *et al.* 1993; Pina *et al.* 2012), highlighting its significance in the study of predatory mites. In the face of climate change, the role of alternative food sources, such as pollen, becomes increasingly vital in mitigating the competitive pressures intensified by the shifting climate. Pollen positively affects the biology of predatory mites to stable populations. For example, applying pollen to vineyards increased predatory mite egg and motile form densities, illustrating the importance of supplemental pollen in sustaining mite populations, particularly during periods when natural pollen is scarce (Malagnini *et al.* 2022).

Urbaneja-Bernat *et al.* (2019) and Urbaneja-Bernat & Jaques (2021, 2022) explored the nuanced effects of future climate conditions on key predatory mites, specifically *E. stipulatus* (Athias-Henriot), *N. californicus*, and *P. persimilis*, which are essential for managing *T. urticae* populations in citrus groves. A central theme in their investigations is the role of alternative food sources, such as pollen, in mitigating the competitive pressures exacerbated by climate change. Such pressures threaten to diminish the natural resilience of these predatory mites in their battle against pests in increasingly warmer futures. Urbaneja-Bernat *et al.* (2019) initially outlined the looming challenges predatory mites are likely to face under future climate scenarios, highlighting potential decreases in biological control efficacy due

to harsher environmental conditions. Urbaneja-Bernat & Jaques (2021, 2022) subsequently provided empirical evidence that access to high-quality pollen sources can significantly enhance the resilience of *E. stipulatus* and *N. californicus*, evidenced by increased survival, oviposition rates, and predation capabilities amidst intensified competition and shifting climate conditions.

These findings underscore the complexity inherent in biological control systems and the need for a nuanced understanding of each species' dietary preferences and life histories. Moreover, the studies emphasize the importance of incorporating adaptive strategies, like providing alternative food sources and leveraging ecological niche modeling, to predict and enhance biological control success under varied climatic conditions. To navigate the complexities introduced by climate change, this case study underscores the necessity for continued research focused on understanding the specific impacts of temperature fluctuations, altered precipitation patterns, and the frequency of extreme weather events on predatory mite populations and their prey dynamics. By integrating these insights, the case study highlights the indispensable role of adaptive management strategies in maintaining the efficacy of biological control agents. It points towards the need for innovative measures, such as the provision of alternative food sources, to mitigate the impacts of climate change on biological control efforts, ensuring the sustainability of agricultural practices for the future.

IDENTIFIED KNOWLEDGE GAPS OF PREDATORY MITES IN CLIMATE CHANGE WORLD

The established utility of predatory mites in biological control, highlighted by the vast biodiversity and adaptability of phytoseiid species (Demite *et al*. n.d.; Chant & McMurtry 2007), stands in stark contrast to the accelerating challenges posed by climate change. This scenario unveils pivotal knowledge gaps, underscoring the need for a comprehensive understanding of climatic thresholds essential for the survival, reproduction, and efficacy of predatory mites.

Exploring indigenous phytoseiid species

Investigating the biodiversity of phytoseiid mites is not merely about cataloging species; it is crucial for uncovering potential indigenous predators with innate adaptability to local climates. Species such as *N. womersleyi* and *N. barkeri* are noted for their superior adaptability to their native climates (Ho 2005; Liao *et al*. 2020a). This adaptability suggests these species possess specific adaptive behaviors, habitat preferences, and stress responses that confer resilience to environmental pressures, including those induced by climate change. Döker *et al*. (2021) emphasize the importance of understanding interactions between indigenous and exotic phytoseiids for effective biological control. A focused examination of these indigenous species can enhance our understanding of their potential as biological control agents and guide the development of climate-resilient strategies. Leveraging the unique traits of indigenous phytoseiids can optimize biological control practices by aligning them with local ecosystem dynamics.

Climatic preferences and impacts on predatory mites

A primary knowledge gap lies in our understanding of the specific climatic preferences of predatory mites and how these preferences impact their role in biological control. While ecological niche modeling has shown potential in identifying regions climatically suitable for biological control agents (Liao *et al*. 2021a, 2023a), the contrasting success rates of species such as *N. californicus* and the challenges encountered by *N. fallacis* and *P. persimilis* in adapting to Taiwan's climate (Liao *et al*. 2021a, 2023a) underscore the need for detailed research into the climatic factors that favor or hinder these mites. This in-

cludes a deeper exploration into species-specific climate preferences, such as optimal temperature and humidity ranges, and how deviations from these conditions affect their survival, reproduction, and efficacy in pest control. Understanding these climatic impacts is crucial for predictive modeling and selecting the most effective mite species for biological control in varying climatic conditions (Thompson *et al*. 2010; Ghazy *et al*. 2016).

Uncovering the genetic foundations of adaptability

Investigating the genetic factors that enable predatory mites to adapt to diverse climatic conditions is essential. Research focused on the molecular responses of these mites to environmental stressors can uncover genetic variations that enhance resilience to climatic changes (Ghazy *et al*. 2016; Zhang *et al*. 2019; Cruz-Miralles *et al*. 2021). Identifying these traits allows for the strategic selection or genetic enhancement of predatory mites, equipping them to better withstand the challenges of climate change (Cruz-Miralles *et al*. 2021). This approach not only promises more effective biological control agents in the face of current climatic variability but also prepares these agents to meet future environmental conditions. By leveraging genetic insights, we can improve the adaptability of both indigenous and introduced species to ensure the long-term efficacy and resilience of biological control strategies.

Transgenerational responses to climate extremes

The impact of extreme climate events such as heat waves on predatory mites and their subsequent generations remains underexplored. Tscholl *et al*. (2023) revealed that exposure to such events can alter reproductive investments in these mites, suggesting a nuanced interaction between climate stressors and biological control effectiveness. How environmental stress impacts mites' physiological responses, which may extend to subsequent generations, remains to be explored (Veerman 1992; Ghazy *et al*. 2016). Additionally, Zhang *et al*. (2016) showed that heat stress affects reproductive success, underscoring the potential for transgenerational impacts. This highlights the need for further research into transgenerational adaptability and resilience of biological control agents to climate extremes. Understanding how these stressors affect not only the immediate generation but also subsequent ones is vital for refining biological control strategies to withstand the challenges posed by a changing climate.

RECOMMENDATIONS FOR FUTURE CLIMATE-ADAPTED BIOLOGICAL CONTROL WITH PREDATORY MITES

Adapting biological control strategies to the challenges posed by global climate change is critical for ensuring the sustainability of agricultural ecosystems. A key initial step involves exploring the diverse habitats of phytoseiid mites to identify potential natural enemies with high adaptability to future climate conditions. This exploration is essential not only for cataloging species but also for uncovering those with innate resilience to varying environmental conditions. Members within the genus *Euseius*, such as *E. stipulatus*, are exemplary in their versatility and resilience, capable of thriving on a range of food sources including pollen, which highlights their potential in climates altered by global warming (McMurtry *et al*. 2013; Cruz-Miralles *et al*. 2021). However, the identification of highly adaptable species is just the beginning. Creating supportive agricultural ecosystems that can sustain these selected biological control agents through every season and climatic challenge is equally important. This includes incorporating indigenous species like *N. womersleyi* and *N. barkeri* into biological control schemes, leveraging the natural biodiversity to address

pest challenges effectively and environmentally sensitively (Ho 2005; Liao *et al.* 2020a). The local climatic adaptation of these species provides a foundation for deploying biological control agents that are most likely to succeed in environmental settings.

It is crucial to consider their potential impact on non-target species and the overall ecosystem when developing biological control strategy (Nechols 2021). This comprehensive approach to biological control underlines the importance of not only enhancing the adaptability and efficacy of biological control agents but also ensuring that these interventions do not adversely affect the biodiversity and ecological balance of agricultural landscapes. Moving forward demands a synergy of research and practical approaches to ensure biological control agents not only survive but thrive under the stress of climate change (Thomson *et al.* 2010; Nechols 2021; Liao *et al.* 2023a). This involves delving into ecological and genetic research to discover how predatory mites adapt to different climates and environmental pressures (Ghazy *et al.* 2016; Cruz-Miralles *et al.* 2021). Utilizing ecological niche modeling is crucial in this context (Liao *et al.* 2023a), refining our predictions about the regional suitability for biological control agents and the changing dynamics of pest populations in response to climate change.

Developing agricultural landscapes that can withstand climate extremes is foundational to this strategy, including the incorporation of domatia, small structures on the undersides of leaves that provide shelter for beneficial organisms like predatory mites (Fig. 3) (Walter 1996). Implementing diversified planting schemes, windbreaks, and shade structures can create microclimates that shelter biological control agents from severe conditions. Moreover, ensuring consistent availability of alternative food sources (Shih *et al.* 1993; Pina *et al.* 2012; Urbaneja-Bernat *et al.* 2019; Urbaneja-Bernat & Jaques 2021, 2022), such as pollen, during periods of low pest activity is vital for maintaining predator populations and extending their biological control effectiveness. Innovations like slow-release sachets further reinforce the presence and impact of biological control agents in the field, contributing to their prolonged effectiveness (Shimoda *et al.* 2017, 2023; Urbaneja-Bernat & Jaques 2022). Backing these practical strategies with collaborative research and policy support for sustainable agriculture practices is crucial for a comprehensive approach to climate-adapted biological control. Integrating research insights with advanced, proactive strategies enables the agricultural sector to adeptly manage the intricacies introduced by climate change, ensuring the continuity of biological control practices. This integrated approach is vital for preserving the balance between agricultural productivity and ecosystem health in an era of significant environmental transformation.

CONCLUSION

This review has underscored the significance of predatory mites in agricultural ecosystems amid the challenges of climate change. It highlights the necessity for adaptive biological control strategies that align with the specific resilience characteristics of predatory mites and the overarching health of ecosystems. The intricate relationships between climate factors and the biological control potential of predatory mites demand a nuanced understanding and strategic ecosystem modifications to enhance their survival and effectiveness (McMurtry *et al.* 2013; Nechols 2021; Liao *et al.* 2023a).

As climate change continues to reshape the dynamics of agricultural pests and their natural enemies, this study advocates for a proactive approach in biological control practices. This entails a dual focus on selecting inherently resilient mite species and implementing ecosystem enhancements to buffer against extreme climatic conditions (Ghazy *et al.* 2016; Urbaneja-Bernat *et al.* 2019). Moreover, the evolving nature of climate change compels ongoing research to refine biological control strategies, ensuring they remain effec-

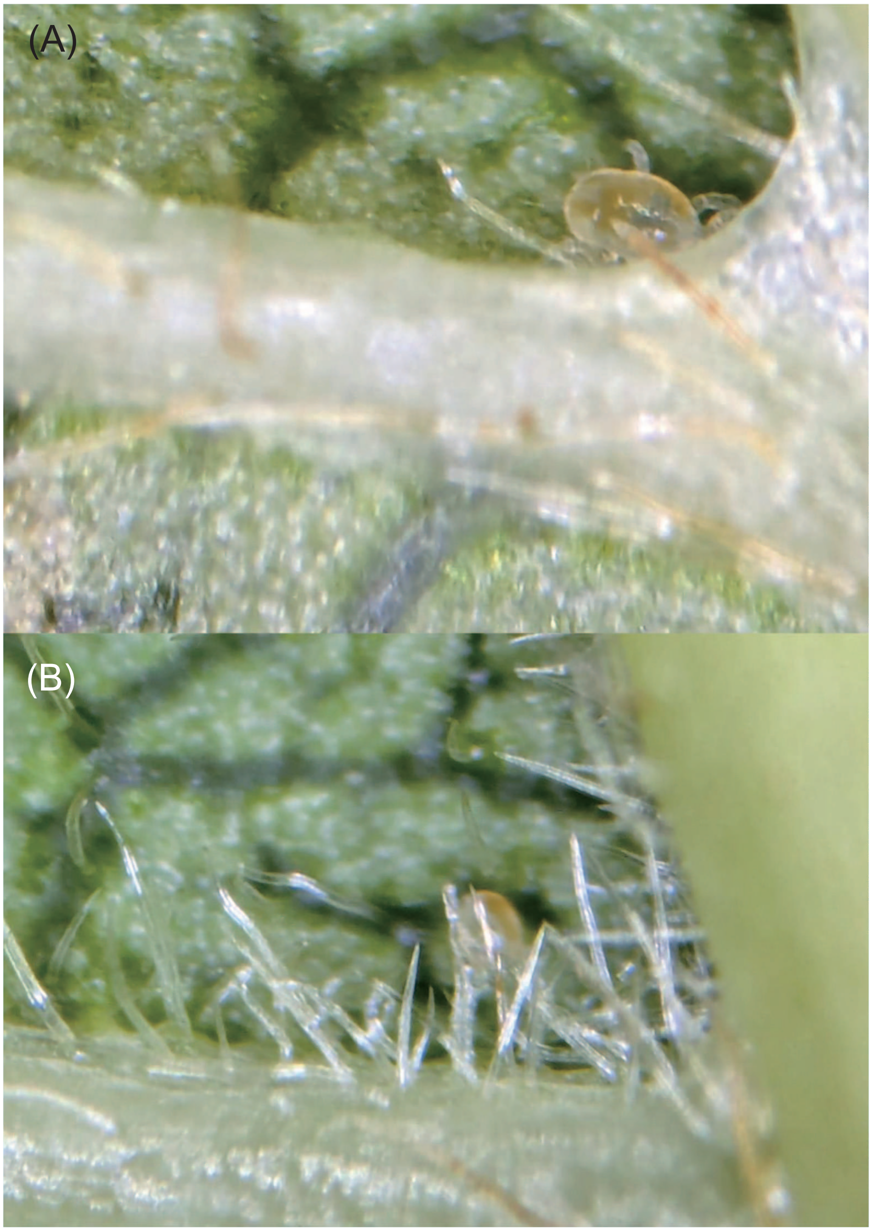

Fig. 3. Predatory mites on domatia on cherry tree leaves. (A) Larva of *Amblyseius* species; and (B) egg of predatory mite.

tive under changing environmental conditions (Thomson *et al*. 2010). The future of biological control in agriculture hinges on our ability to innovate and adapt. By prioritizing research and practices that enhance the adaptability of predatory mites, we can ensure the sustainability of biological control as a fundamental component of integrated pest management in the face of global climate change.

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氣候變遷下捕植蟎生物防治的挑戰與未來方向

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摘要

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氣候變遷對農業生態系統造成了重大的挑戰，尤其是在害蟲、天敵動態及生物防治的有效性方面。捕植蟎 (植綏蟎) 作為捕食多種農業害蟲的重要捕食性天敵，面臨著氣候變遷帶來的威脅。本文回顧了氣候變遷對捕植蟎生物防治潛力的影響，並指出了目前研究中存在的關鍵知識缺口，這些缺口制約了發展出適應氣候變遷的生物防治策略。文中深入探討了氣候變遷，如溫度波動、降水模式變化及極端氣候事件，對捕植蟎的生理狀態、分布及其與害蟎互動的影響。透過案例研究，本文展示了在變化氣候條件下管理捕植蟎具體實踐與適應措施。本文強調了針對物種特定氣候適應性進行深入研究的必要性，並呼籲加強生物防治結果的預測模型。為應對氣候變遷帶來的挑戰，創新的管理實踐至關重要。本綜述強調，需要結合生態學、遺傳學及技術見解的綜合方法，來維持全球氣候變遷下生物防治系統的功能。

關鍵詞：氣候韌性、害蟲管理、適應策略、生態系統動態、可持續農業。

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Research Article

The Bacterial Stalk Rot of Maize Caused by *Dickeya oryzae* in Taiwan

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Abstract

Tsai, C. H., S. L. Hwang, J. R. Ku, M. J. Lin, C. Y. Lin, C. P. Kuan, and C. C. Chen. 2024. The bacterial stalk rot of maize caused by *Dickeya oryzae* in Taiwan. J. Taiwan Agric. Res. 73(3):169−180.

In 2017, water-soaked brown lesions were found in the stalk of the maize plants in Yuanchang and Huwei township, Yunlin County. The stalks were finally softened by the spreading lesions, resulting in hollowing and lodging of the plants. The bacteria were isolated from the diseased stalk tissues and cultured on nutrient agar. The pathogenicity of the bacteria was verified by Koch's postulates. The 16S rDNA, *gyrB* and *dnaX* gene sequences of the pathogen showed a high identity to *Dickeya zeae*, and multilocus sequence analysis revealed that the pathogen, *D. zeae* and *D. oryzae* were grouped as a clade. However, a publication of a new species of *D. oryzae* reassigned some *D. zeae* strains to *D. oryzae*, and the pathogen was further identified as *D. oryzae* based on the average nucleotide identity nucleotide of whole genome sequences in our study. This is the first report of maize bacterial stalk rot caused by *D. oryzae* worldwide. In the host range test, the pathogen could infect potatoes, carrots, onion bulbs, Welsh onions, rice, and cabbages; it therefore showed a potential threat to the agricultural industry. On screening agrochemicals, the 500-fold-diluted 20% oxolinic acid showed the most effective inhibition of the pathogen growth.

Key words: Maize, Bacterial stalk rot, *Dickeya oryzae*.

INTRODUCTION

Maize (*Zea mays* L.) is an important miscellaneous grain crop, having the largest planting area in Taiwan. It can be used as feed and food. According to the 2022 Taiwan Agricultural Statistics Annual Report (https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx), the planting area of feed maize crops was 20,148 ha, and the main production areas were Tainan City and Chiayi County. The planting area of the edible maize crops was 15,067 ha, with their main production area located in Yunlin County, Tainan City, Chiayi County, and Hualien County.

Many bacterial diseases of maize have been found in Taiwan, including bacterial stripe disease (pathogens *Acidovorax avenae* subsp. *avenae* and *Burkolderia andropogonis*), and bacterial soft rot (pathogens *Erwinia chrysan-*

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themi and *E. carotovora* subsp. *carotovora*) (Tzean *et al*. 2019). In addition, Stewart's wilt (pathogen *Pantoea stewartii*), Goss's wilt (pathogen *Clavibacter michiganensis* subsp. *nebraskensis*), bacterial stalk rot (pathogen *Dickeya zeae*) (Jardine & Claflin 2016; Kumar *et al*. 2017), etc. were the international bacterial diseases causing maize wilt.

In 2017, some maize plants were observed to appear water-soaked, with brown lesions on the leaf sheath and stalk of the plants in Yunlin County, Taiwan. The brown lesions expanded and eventually softened the stalks. When cutting the stalk tissues of the diseased plant and observing it under a microscope, a large number of bacterial streaming out of the tissue was observed, which is suspected to be bacterial disease. Since the incidence of the disease in the field was about 10%, and there were no recommended agrochemicals to control the disease, the causal agent of this maize disease was identified in this study. In addition, the effects of agrochemicals on inhibiting bacterial growth were evaluated in the laboratory for future application in field control.

MATERIALS AND METHODS

The source of bacterial isolates

Diseased maize plants were collected from different fields in Huwei Township and Yuanchang Township, Yunlin County. The symptoms of water-soaked and brown lesions were observed on the leaf sheath and stalk of the maize plants (Fig. 1A). Then the brown lesions further expanded and spread on the stalk (Fig. 1B). The vascular bundles showed browning when the diseased stalks were cut longitudinally (Fig. 1C). Later, the stalks of the plants were hollowing, wilt, and toppled down. The infected ears of maize were brown and softened, which would reduce the commercial value of maize (Figs. 1D–E).

The maize plants with water-soaked and softened symptoms were collected from different fields. The diseased tissues of the plant were cut out and sterilized in 0.5% sodium hypochlorite and then rinsed with sterile water 3 times. The tissues were further minced in sterile water to release the bacteria. The bacterial suspension was dipped with the loop and spread on the nutrient agar (NA) medium (Difco Laboratories; Becton, Dickinson and Company, Le Pont-de-Claix, France). The NA medium containing the bacteria was placed in a 30℃ incubator for cultivation. The single colony of cultured bacteria was transferred to a new medium for purification. The purified bacteria were made into a bacterial suspension and injected into tobacco leaves, when the necrotic spots appeared after 1 d, indicating that the bacteria might be pathogenic. Therefore, the pathogenic bacteria from different fields were numbered and preserved for further identification and subsequent experiments. Two bacterial isolates, BSRM01 and BSRM02 were collected from Huwei Township, and the BSRM03 isolate was collected from Yuanchang Township.

Biolog identification of the bacteria

Three bacterial isolates, BSRM01, BSRM02, and BSRM03, collected from different fields were purely cultured on the BUG™ medium (Biolog Universal Growth Agar, Biolog Inc., Hayward, CA, USA) in a 30℃ incubator for 16−24 h. The single colony was picked up with a cotton swab and bacterial cells were suspended in IF-A inoculum (Biolog Inc., Hayward, CA, USA). The concentration of bacterial suspension was adjusted to 90−98% T (turbidity) with a turbidimeter, at a wavelength of 590 nm. The bacterial suspension was inoculated into the Biolog GEN III plates (Biolog Inc., Hayward, CA, USA), and each well of plates was inoculated into 100 μL inoculum. The inoculum GEN III plates were incubated for 8−24 h, and the color change was measured with a spectrometer. The reading values of the plate were analyzed using Biolog MicroLog™ 3 ver.5.22 system (database version 2.6.1).

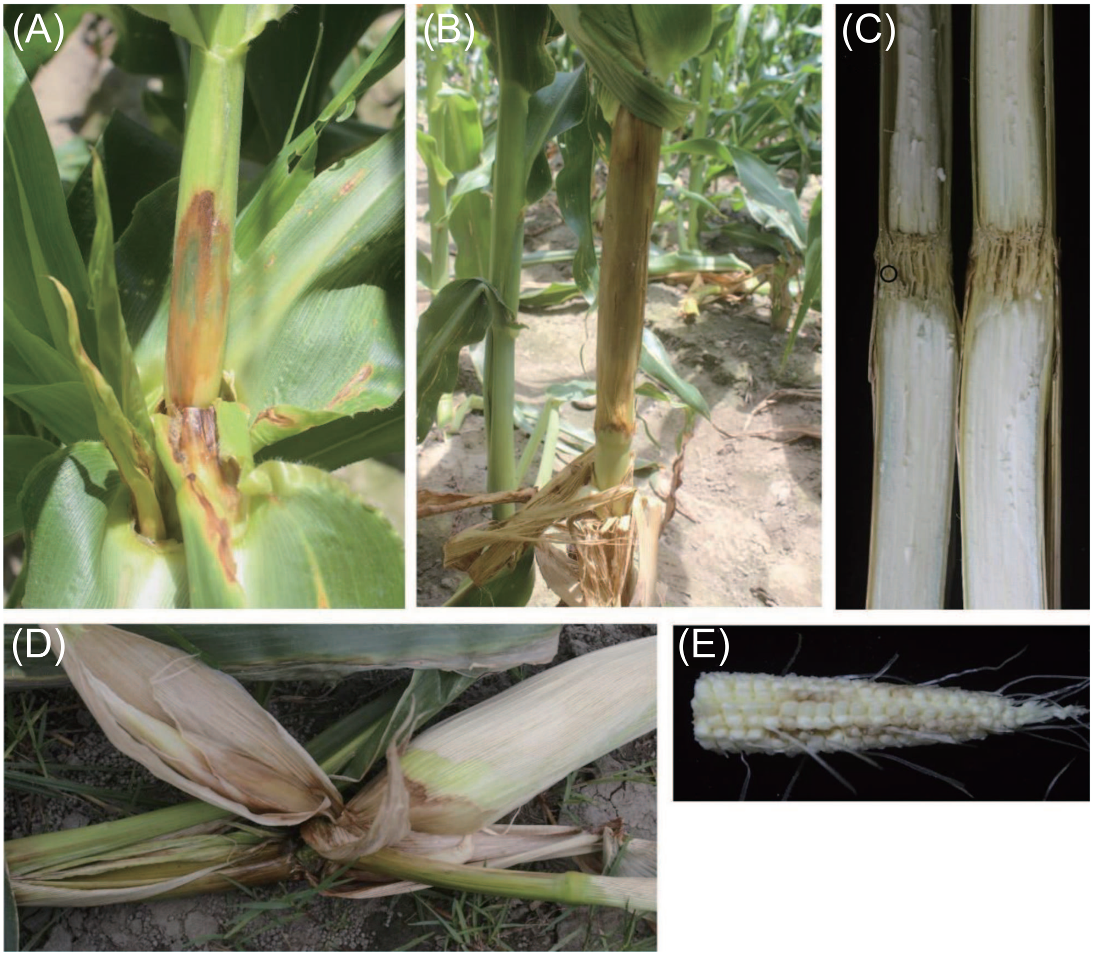

Fig. 1. Symptoms of maize bacterial stalk rot observed in the field. (A) Water-soaked blotch on the surface of the stalk at the initial stage of the disease; (B) browning on the stem surface; (C) browning symptom in vascular bundle of the stem; (D) water-soaked browning of the diseased maize ears; and (E) browning and soft rot symptoms on maize kernels.

PCR and gene cloning for sequencing

Three bacterial isolates, BSRM01, BSRM02, and BSRM03, were purely cultured on NA medium. The extraction of the bacterial total nucleic acid procedure described by Wang *et al*. (1993) was slightly modified as follows. The bacterial single colony of 3 bacterial isolates was individually dipped with a loop into 20 μL of sterile water to be bacterial suspension. The 20 μL of 0.4 N NaOH was added to the suspension and mixed well for 10 min, and 40 μL of 1 M Tris-HCl (pH = 8.0) was subsequently added to the mix for neutralization. Then 20 μL of suspension was pipetted for 10× dilution with sterile water to serve as a DNA template.

The 16S rDNA, gyrase subunit B gene (*gyrB*), and DNA polymerase Ш gamma subunit gene (*dnaX*) partial sequences were amplification by PCR using the 16S rDNA universal primer pair f8-27/r1510 (Lipson & Schmidt 2004), the specific primer pair gyrBf1/gyrBr1 (Pu *et al*. 2012) for *gyrB* and the specific primer pair dnaXf/dnaXr (Sławiak *et al*. 2009) for *dnaX*. The PCR amplicons were analyzed on 1.4% agarose gel. The obtained expected DNA fragments were cloned with a T&A^{TM} cloning

kit (Yeastern Biotech, Taipei, Taiwan), and the successfully cloned DNA fragments were sent to the biotech company for DNA sequencing. The obtained 16S rDNA (NCBI accession number ON430645), *gyrB* (NCBI accession number ON462306), and *dnaX* (NCBI accession number ON462307) sequences were deposited at GenBank and BLASTn in National Center for Biotechnology Information (NCBI).

Multilocus sequence analysis (MLSA)

The BSRM01 was selected as a representative isolate for MLSA. Partial sequences of the 3 housekeeping genes 16S rDNA, *gyrB*, and *dnaX* of BSRM01 and the gene sequences of reference *Dickeya* sp. downloaded from NCBI were used for MLSA. The DNA sequences were aligned with Clustal W (Larkin *et al*. 2007) and further constructed a phylogenetic tree using neighbor-joining analysis with 1,000 bootstrap replicates by MEGA11 software (Tamura *et al*. 2021).

Whole genome DNA sequence analysis

The bacterial isolate BSRM01 was purely cultured in 5 mL of nutrient broth for 24 h. The cultured bacterial suspension was extracted with the nucleic acid extraction kit (EasyPure stool genomic DNA kit) for total nucleic acid extraction. The total nucleic acids of bacteria were sent to a biotechnology company (Tri-i Biotech, Inc.) for Illumina MiSeq paired-end sequencing, and the sequences of short fragments were assembled using the SPAdes assembly software (Version 3.15.3) for de novo draft genome sequence. The draft whole genome sequence of bacterial isolate BSRM01 obtained by assembly estimated the average nucleotide identity (ANI) values with *D. zeae* EC1 strain (GenBank accession CP006929.1), *D. oryzae* type strain ZYY5^T (GenBank accession SULL00000000) and *D. zeae* type strain NCPPB2538^T (GenBank accession CM001977.1), respectively, using orthologous average nucleotide identity (OrthoANI) (Lee *et al*. 2016).

Koch's postulates test

Three bacterial isolates, BSRM01, BSRM02, and BSRM03 were used in this test. After the bacteria were cultured on a nutrient agar medium plate for 2 d, the bacteria cells were suspended in sterile water, and measured with a spectrophotometer (spectrophotometer, Spectronic 70, Bausch & Lomb, Bridgewater, NJ, USA) at a wavelength of 600 nm. The bacterial suspension was adjusted to an absorption value of 0.3 (concentration is about 10^8 cfu mL^{-1}) as the inoculation source. The test plants "Yumeizhen" maize were planted in a pot about 50 cm high. The inoculation method was to put a drop of bacterial suspension first 10 μL on the stalk, and the stalk was then punctured with a sterile needle to create a wound for bacterial infection. Each bacterial isolate was inoculated with 3 plants. The control plants were treated with sterile water. After inoculation, the plants were kept moist in plastic bags for 24 h, and then the plastic bags were opened and placed in a growth chamber at 30℃ for observation of disease symptoms. The pathogenic bacteria were re-isolated from the stalks of the diseased plants, and the isolated bacteria were confirmed to be the same as the inoculated isolates.

Pathogenicity of the bacteria to different crops

In order to understand the host range of the bacterial isolate, different crops referred to Lin *et al*. (2016) were inoculated with 3 bacterial isolates, BSRM01, BSRM02, and BSRM03, for the inoculation test of various crops. The inoculated crops included rice plants, potato tubers, Welsh onions, carrot roots, onion bulbs, and cabbages; they were kept moist for 2 d after inoculation, and the symptoms of each crop were observed daily.

Laboratory agrochemicals susceptibility test

The paper disc diffusion method (Adaska-

veg & Hine 1985) was used to test the effectiveness of several commercially available agrochemicals in inhibiting the bacterial pathogen of maize stalk rot.

BSRM01 (from Huwei Township) and BSRM03 (from Yuanchang Township) were selected as test isolates. The tested agrochemicals were selected from the plant protection handbook, and their testing concentration range was slightly adjusted according to the suggested concentration in the handbook. Four types of agrochemicals were used: (1) antibiotics, such as streptomycin + tetracycline (10.0% water soluble powder; SP), streptomycin (12.5% soluble concentrate; SL), kasugamycin (2.0% SL), oxolinic acid (20.0% wettable powder; WP), and validamycin (10% SL); (2) copper-containing agrochemicals, such as copper hydroxide (53.8% water dispersible granule; WG), copper oxychloride (85.0% WP), and tribasic copper sulfate (27.12% suspension concentrate; SC); (3) zinc-manganese-containing agrochemical such as mancozeb (80.0% WP); (4) mixed agrochemical, such as thiophanate methyl + streptomycin (68.8% WP), kasugamycin + copper oxychloride (81.3% WP).

The agrochemical testing method was as follows: first, prepare a bacterial suspension and adjust the concentration to approximately 10^8 cfu mL^{-1}, add 0.1 mL of bacterial suspension to 6 mL of water agar, mix well, and then cover it with NA medium. Add 0.12 mL of each agrochemical diluted to different concentrations into a filter paper disk with a diameter of 13 mm (Whatman International Ltd., Chalfont St. Giles, UK), and then place the filter paper disk containing the agrochemicals on the NA medium covered with bacterial water agar. The filter paper disc dripped with sterile water was used as a control treatment, and each treatment was repeated 3 times. Afterwards, the culture plates of each treatment were placed in a constant temperature oven at 28℃ for 48 h, and then the diameter of the inhibition zone (minus filter paper diameter 13 mm) was measured to determine the effect of agrochemicals.

RESULT AND DISCUSSION

Identification of bacteria by Biolog system

Three bacterial isolates, BSRM01, BSRM02, and BSRM03, from different fields were analyzed by the Biolog identification system to determine the physiological characteristics and the utilization of carbon sources. The bacterial isolates were cultured at 30℃ for 16−24 h, and then the color change of the reaction plate was measured with a spectrometer. The measured readings were compared with the database by Biolog MicroLog™. These 3 bacterial isolates, BSRM01, BSRM02, and BSRM03, were most similar to *D. chrysanthemi*. Their similarity values were 0.638, 0.646, and 0.638, respectively, exceeding the critical value of 0.5.

D. chrysanthemi was originally *Pectobacterium chrysanthemi*. Samson *et al*. (2005) established the genus *Dickeya* and reclassed *P. chrysanthemi* to 6 new species, namely *D. chrysanthemi*, *D. dadantii*, *D. dianthicola*, *D. dieffenbachiae*, *D. paradisiaca*, and *D. zeae*. Since then, some new species of *Dickeya* have been published including *D. aquatic* (Parkinson *et al*. 2014), *D. solani* (van der Wolf *et al*. 2014), *D. fangzhongdai* (Tian *et al*. 2016), *D. lacustris* (Hugouvieux-Cotte-Pattat *et al*. 2019), *D. undicola* (Oulghazi *et al*. 2019), *D. poaceiphila* (Hugouvieux-Cotte-Pattat *et al*. 2020), *D. oryzae* (Wang *et al*. 2020), *D. parazeae* (Hugouvieux-Cotte-Pattat & Van Gijsegem 2021). However, the database of Biolog MicroLog™ didn't contain all the *Dickeya* sp. It was necessary to further identify the bacteria species.

Molecular identification of pathogenic bacteria

The 16S rDNA, *gyrB*, and *dnaX* genes of the representative bacterial isolate BSRM01 from the gene cloning were sequenced. The obtained 16S rDNA sequence, *gyrB*, and *dnaX* were deposited to GenBank and BLASTn in the NCBI gene database. The results showed that

these 3 gene sequences had the highest identity with the sequence of *D. zeae* strains, reaching more than 99%. MLSA was performed on the 3 genes, and a phylogenetic tree was constructed. The bacterial isolate BSRM01, *D. zeae* and *D. oryzae* ZYY5 reference strains were assigned to a clade (Fig. 2).

D. zeae was originally known as *P. chrysanthemi* pv. *zeae*. Samson *et al*. (2005) reclassed *P. chrysanthemi* pv. *zeae* to *D. zeae*. In 2020, a new species of *D. oryzae* was published according to the differences in the whole genome sequence, and some strains originally belonging to *D. zeae*, such as *D. zeae* EC1 strain,

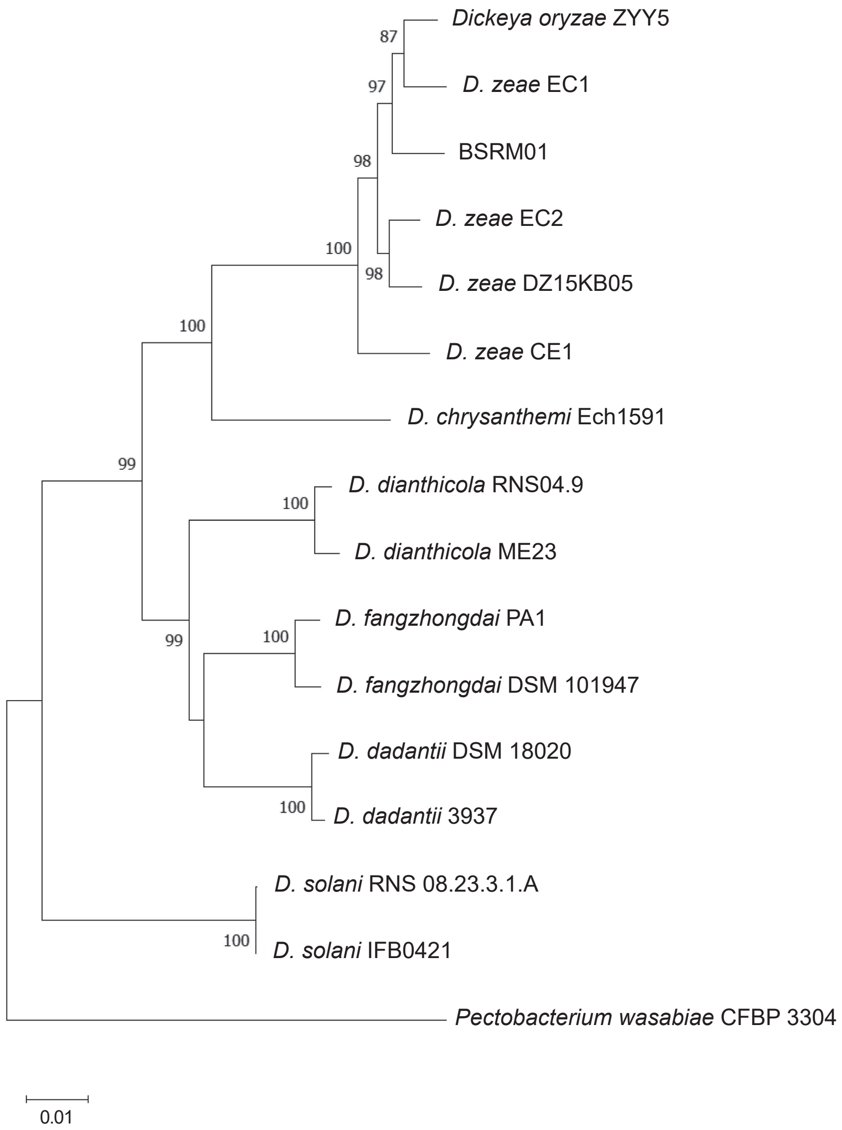

Fig. 2. A neighbor-joining phylogenetic tree of BSRM01 isolated from maize stalk rot plant and reference strains, based on partial 16S rDNA, DNA gyrase subunit B gene, and DNA polymerase Ш gamma subunit gene sequences. The number on the branches represents bootstrap values. The scale bar indicates 1 nucleotide change per 100 nucleotides. Bootstrap values are indicated at branch points based on 1,000 replications. Bootstrap values below 60% are not shown.

should be reassigned as *D. oryzae* (Wang *et al.* 2020). The MLSA result indicated that the bacterial isolate BSRM01 was very close to *D. zeae* EC1 and *D. oryzae* ZYY5^{T}. The bacterial isolate BSM01 should be further analyzed by whole gene sequencing to clarify its species status.

Whole genome DNA sequence analysis of the bacterial isolates

From the above, the bacterial isolate BSRM01 was very close to *D. zeae* EC1 and *D. oryzae* based on the phylogenetic analysis of MLSA (Fig. 2). However, *D. zeae* EC1 was suggested to be reclassed to *D. oryzae*. In order to clarify the taxonomic status of the bacterial isolates, the extracted total nucleic acid of bacteria was sent to a biotechnology company for whole-genome sequencing. The genome was sequenced using the Illumina MiSeq pair-end sequencing system, and the sequences of short fragments were assembled using SPAdes assembly software (Version 3.15.3).

The assembled genome sequence of the BSRM strain was 4,959,956 bp long, with 42 contigs, an N50 of 324,712 bp, and an average coverage of 177.8.

The assembled draft genome sequence of the bacterial isolate BSRM01 (NCBI accession number JBCGHM000000000) was compared with *D. zeae* type strain NCPPB2538^{T}, *D. zeae* EC1 and *D. oryzae* type strain ZYY5^{T} for calculation of ANI. The results showed that the identity of the isolate BSRM01 shared 94.61% with *D. zeae* NCPPB2538^{T}, 95.58% with *D. zeae* EC1, and 95.8% with *D. oryzae* ZYY5^{T}. The ANI values of BSRM01 with *D. oryzae* ZYY5^{T} and *D. zeae* EC1 were more similar than BSRM01 with *D. zeae* NCPPB2538^{T}.

Wang *et al.* (2020) established new species of *D. oryzae* and reassigned *D. zeae* EC1 as *D. oryzae*. The generally accepted species boundary for ANI values is 95−96% (Lee *et al.* 2016). The ANI value of BSRM01 was 95.8% with *D. oryzae* ZYY5^{T} and 95.58% with *D. zeae* EC1, both of which were in the species boundary 95−96%. Therefore, the BSRM01 strain was classified as *D. oryzae*.

Koch's postulates test

Bacterial isolates BSRM01, BSRM02 (isolated from Huwei Township), and BSRM03 (isolated from Yuanchang Township) were purely cultured on the nutrient agar and made into a bacterial suspension with a concentration of about 10^{8} cfu mL^{-1}. The bacterial suspension was inoculated on the 'Yumeizhen' maize plant with the puncture stem method. After 2 d of inoculation, the maize plant appeared water-soaked at the inoculation site, and then the stalk showed symptoms of browning and softening. The maize plants gradually wilted and toppled down after 7 d. When the stalk of diseased plants was cut longitudinally, the vascular bundles showed obvious browning. The symptoms of the inoculated plants were the same as those seen in the field. The plants inoculated with sterile water were symptomless, as shown in Fig. 3. The same pathogenic bacteria could be isolated from the stalk of the diseased plants after inoculation.

Pathogenicity of the bacteria to different hosts

The results of different plants inoculated with the tested bacterial isolates BSRM01, BSRM02, and BSRM03 revealed that the bacteria could infect several economically important crops. Three days after the inoculation, the 3 bacterial isolates caused obvious symptoms of tissue softening and browning on rice, potato, carrot, onion bulb, and Welsh onion (Fig. 4). However, the pathogenicity to Chinese cabbage was relatively weak, and black-brown necrotic spots were limited at the inoculation site (Fig. 4D).

D. oryzae previously known as *D. zeae* (Wang *et al.* 2020). *D. zeae* could infect various plants and cause soft rot symptoms in crops and ornamental plants, such as maize, rice, pineapples, chrysanthemums, potato, banana tobacco, tomatoes, eggplants, peppers and clivia (Samson *et al.* 2005; Kumar *et al.* 2017; Hu *et al.* 2018). In Taiwan, Lin *et al.* (2016) reported that *D. zeae* caused rice bacterial foot

Fig. 3. Symptoms of the maize plants after artificial inoculation with bacterial isolates BSRM01, BSRM02, and BSRM03. (A) The plant showed wilting symptoms at 7 d after inoculation (left), and plants showed symptomless when inoculated with sterile water (right). (B) Close-up of the vascular browning symptoms.

rot and could infect several economic crops through inoculation tests. Our host range test of *D. oryzae* was similar to *D. zeae* described by Lin *et al*. (2016), showing that *D. oryzae* is a potential threat to various economic crops in the agricultural industry.

Susceptibility of agrochemicals tested in laboratory

Disease control of bacterial stalk rot of maize has no recommended agrochemicals at present. However, when the environment is suitable for the occurrence of this disease, it may cause significant losses to farmers. Therefore, it is important to evaluate those commercial agrochemicals for reference in disease control. The growth inhibitory effects of 10 agrochemicals listed in the plant protection manual were tested by the filter paper disc diffusion method. By measuring the diameter of the inhibition zone, the 4 treatments of agrochemicals, consisting of oxolinic acid, streptomycin + tetracycline, streptomycin, and thiophanate-methyl + streptomycin, yield effects on the growth inhibition of the bacterial isolates. Among these treatments, the oxolinic acid treatment produced the largest inhibition zone (Table 1). These results can be applied in subsequent greenhouse and field control experiments.

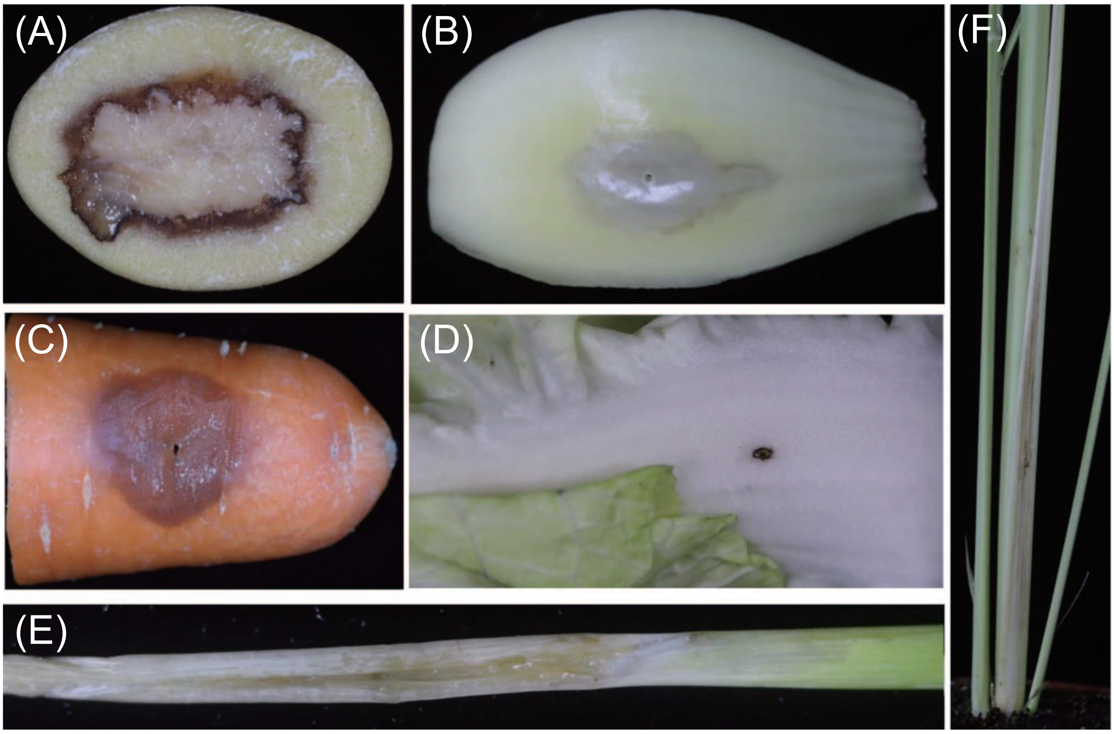

Fig. 4. Symptoms of different crops inoculated with maize bacterial isolates BSRM01, BSRM02, and BSRM03. (A) Browning soft symptom showed on potato slices at 4 d after inoculation; (B) water soaking and soft rot symptoms showed on onions at 4 d after inoculation; (C) browning soft symptom showed on carrots at 3 d after inoculation; (D) necrotic spot symptoms showed on Chinese cabbages at 7 d after inoculation; (E) soft rot symptom showed on shallots at 7 d after inoculation; and (F) browning symptom showed on rice seedlings at 4 d after inoculation.

CONCLUSION

In 2017, some maize plants showed water-soaked and soft rot symptoms on stalks in the fields of Yunlin County. These symptoms were similar to bacterial stalk rot of maize described in the literature (Kumar *et al*. 2017). The bacteria isolated from diseased maize tissues were confirmed as the causal pathogen through Koch's postulates test and further identified as *D. oryzae* through gene sequence BLASTn, MLSA and ANI analysis. This is the first report of maize bacterial stalk caused by *D. oryzae* in Taiwan.

D. oryzae originally belonged to *D. zeae*. *D. zeae* was known to cause rice bacterial root rot in Taiwan and distributed throughout Taiwan (Lin *et al*. 2016). Our host range test showed that *D. oryzae* was similar to *D. zeae* and was a potential threat to the agricultural industry. There were currently no recommended agrochemicals for controlling this disease. We tested 10 commercial agrochemicals for their effects in inhibiting bacterial growth and screened out 4 agrochemicals to provide reference for further disease control.

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Table 1. Growth inhibition of *Dickeya oryzae* by various agrochemicals at different concentrations.

Chemical[z]	Dilution fold	Inhibition zone (mm in diameter)	
		BSRM01	BSRM03
Oxolinic acid (20% WP)	500	6.93 ± 0.244 a[y]	7.43 ± 0.12 a
	1,000	5.70 ± 0.21 b	5.70 ± 0.20 c
	2,000	5.13 ± 0.12 c	4.97 ± 0.23 d
Streptomycin + tetracycline (10% SP)	500	5.97 ± 0.12 b	7.07 ± 0.03 b
	1,000	3.80 ± 0.36 e	5.53 ± 0.27 c
	2,000	3.37 ± 0.27 f	4.37 ± 0.13 e
Streptomycin (12.5% SL)	500	4.83 ± 0.13 c	4.50 ± 0.31 e
	1,000	3.80 ± 0.17 e	3.53 ± 0.15 f
	2,000	3.33 ± 0.15 f	3.03 ± 0.09 g
Thiophanate methyl + streptomycin (68.8% WP)	500	4.23 ± 0.12 d	3.73 ± 0.12 f
	1,000	2.77 ± 0.33 g	3.07 ± 0.03 g
	2,000	2.57 ± 0.32 g	2.63 ± 0.18 h
Kasugamycin (2.0% SL)	125	0 h	0 i
	250	0 h	0 i
	500	0 h	0 i
Kasugamycin + copper oxychloride (81.3% WP)	500	0 h	0 i
	1,000	0 h	0 i
	2,000	0 h	0 i
Copper hydroxide (53.8% WG)	750	0 h	0 i
	1,500	0 h	0 i
	3,000	0 h	0 i
Copper oxychloride (85.0% WP)	200	0 h	0 i
	400	0 h	0 i
	800	0 h	0 i
Tribasic copper sulfate (27.12% SC)	250	0 h	0 i
	500	0 h	0 i
	1,000	0 h	0 i
Mancozeb (80.0% WP)	250	0 h	0 i
	500	0 h	0 i
	1,000	0 h	0 i
LSD		0.41	0.31

[z] WP: wettable powder; SP: water soluble powder; SL: soluble concentrate; SC: suspension concentrate; LSD: Fisher's protected least significant difference test.

[y] Mean ± standard error (n = 3). Means followed by the same letter in the same column are not significantly different at 5% level by LSD test.

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Dickeya oryzae 引起之玉米細菌性莖腐病

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摘要

蔡佳欣、黃淑苓、古家榮、林玫珠、林靜宜、關政平、陳金枝。2024。*Dickeya oryzae* 引起之玉米細菌性莖腐病。台灣農業研究 73(3):169–180。

2017 年在雲林縣元長鄉與虎尾鎮的玉米田區，部分植株出現莖部水浸狀病斑，之後莖部隨著病斑上下蔓延而軟化，導致植株空心與倒伏。從罹病莖部組織可在營養瓊脂上分離出 1 種細菌，該細菌之病原性可經過科霍氏法則得到驗證。該病菌以 16S rDNA、*gyrB* 及 *dnaX* 基因序列比對，與 *Dickeya zeae* 具有高度相同性，以多基因序列分析 (multilocus sequence analysis)，顯示病菌與 *D. zeae* 及 *D. oryzae* 歸屬於同一群。由於 *D. oryzae* 新種的發表，一些 *D. zeae* 分離株被重新歸屬為 *D. oryzae*，因此將該病菌進一步經全基因體序列定序，以平均核苷酸相似度 (average nucleotide identity) 分析，將該病菌鑑定為 *D. oryzae*。本文為世界上 *D. oryzae* 引起玉米細菌性莖腐病之首次報告。在寄主範圍測試，該病菌除玉米外，尚可感染馬鈴薯、胡蘿蔔、洋蔥、青蔥、水稻及白菜，對農產業顯示出潛在的威脅。在室內藥劑篩選試驗，以 20% 歐索林酸稀釋 500 倍對該病菌的生長的抑制效果最佳。

關鍵詞：玉米、細菌性莖腐病、細菌性莖腐病菌。

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Research Article

Gut Microbiota of 3 Beetle Larvae and Their Potential for Humic Acid Transformation

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Abstract

Wang, T. C., C. Y. Yeh, Y. C. Lin, B. W. Lin, M. C. Yao, and S. C. Chang. 2024. Gut microbiota of 3 beetle larvae and their potential for humic acid transformation. J. Taiwan Agric. Res. 73(3):181−196.

With the rise in global temperature, the removal and long-term sequestration of CO_2 from the atmosphere have become a shared global goal. Agricultural waste can be transformed by microorganisms into humic substances (HS) that are less prone to decomposition in soil, thereby increasing soil carbon sequestration. This study analyzed the gut microbiota of larvae from 3 different beetle species with varying diets. The gut microbiota of larvae from *Trypoxylus dichotomus*, which feed on decaying wood, were found to be richer and harbored a greater diversity of bacterial species compared to those of *Alphitobius diaperinus* and *Araecerus fasciculatus*. Further analysis of the gut microbiota isolated from 3 beetle larvae species examined the activity of enzymes involved in humic acid biosynthesis, including cellulase, ligninase, laccase, and tyrosinase. Among them, *Bacillus megaterium* BM01 and *B. aryabhattai* BA01 exhibited activities for all 4 enzymes, while *B. subtilis* BS01 showed activities for the first 3 enzymes. To further examine the ability of strains to convert rice straw into humic acid, *B. megaterium* BM01, *B. aryabhattai* BA01, and *B. subtilis* BS01 exhibited an increased humic acid conversion efficiency of 2.4%, 2.3%, and 2.1%, respectively, compared to the control group without inoculation. These findings suggest potential applications in on-site conversion and decomposition of residual rice straw post-harvest to humic acid, thereby enhancing soil carbon sequestration.

Key words: Beetle, Gut microbiota, Humic acid, Soil carbon sequestration.

INTRODUCTION

Human industrial activities have transitioned significantly towards the consumption of fossil fuels since the Industrial Revolution in the 18th century, leading to a steady increase in greenhouse gas (GHG) concentrations such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) in the atmosphere. According to the Sixth Assessment Report (AR6) released by the Intergovernmental Panel on Climate Change (IPCC) in 2023, the atmospheric CO_2 concentration has reached its highest point in 2 million years. Between 2011 and 2020, the average global temperature increased by 1.09℃ compared to the period from 1850 to 1900. Among the contributors to this temperature rise, CO_2 accounted for the highest warming

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effect at 0.79℃, followed by CH_4 at 0.5℃. The assessment report also clearly indicates a nearly linear relationship between cumulative anthropogenic CO_2 emissions and global temperature rise (Lee *et al*. 2023). As global temperature rises, the frequency and intensity of extreme weather events are becoming more severe. Removing CO_2 from the atmosphere and storing it long-term have become a common goal for humanity. In agricultural environments, increasing soil carbon sink capacity stabilizes carbon in the soil, mitigates climate change, offers various benefits such as soil quality restoration, ecosystem functioning, and enhances water and nutrient retention, sustainable agricultural practices, and food security (Lal 2008; Lal *et al*. 2015).

Humic substances (HS) in soil exhibit properties such as recalcitrance, enhancing soil aggregation, and prolonging soil carbon retention (Hassett *et al*. 1987; Fortun *et al*. 1990; Spaccini *et al*. 2002). Agricultural organic waste can be transformed into HS by microbial conversion. This conversion process involves the degradation of organic matter followed by polymerization into HS, with humic acid (HA) and fulvic acid (FA) being the main components (Guo *et al*. 2019). Taiwan generates approximately 5 million tons of agricultural residues annually, with rice straw accounting for 1.5 million tons. After harvest, rice straw is often left in fields requiring labor-intensive and time-consuming management. Utilizing these plant residues on-site to convert them into HS capable of enhancing soil carbon sequestration could contribute significantly to soil carbon storage (Martens 2000).

The diverse species, large populations, and various diets of insects contribute to the rich symbiotic bacterial ecology within their intestines. Intestinal symbiotic bacteria assist hosts in food digestion, detoxification, resistance against various insect pathogens, and maintenance of basic insect survival functions (Jang & Kikuchi 2020). It is known that plant substrates, after being consumed and digested by insects, can be converted to feces rich in HA. For instance, larvae of the white-spotted flower chafer (*Protaetia brevitarsis* Lewis) efficiently convert consumed plant residues into feces with high levels of HA (Li *et al*. 2019). While the exact mechanism of formation remains unclear, studies have found that dominant bacterial strains in insect guts play a role in decomposition and transformation. These strains possess the ability to degrade hemicellulose and cellulose and aid in the decomposition of plant debris (Lou *et al*. 2022). The formation of HS involves the degradation of lignin, followed by the action of tyrosinase and laccase, which polymerize small phenolic compounds, amino acids, and aromatic compounds to form stable molecular structures (Gerke 2018; Piccolo *et al*. 2018). Furthermore, gut bacteria in the alkaline and anaerobic environments of insect intestines can transform ingested organic matter, suggesting a link between HA biosynthesis and gut bacteria (Huang *et al*. 2010; Hobbie *et al*. 2012). Many studies have utilized microorganisms to promote humic substance biosynthesis, such as the use of the white rot fungus *Phanerochaete chrysosporium* and the actinomycete *Streptomyces badius* to degrade lignin in rice straw and convert it into HA and FA (Huang *et al*. 2008). The addition of *Bacillus aryabhattai* to discarded coconut fiber substrates enhances humic substance biosynthesis through the enzymatic activities of tyrosinase and laccase, consequently reducing ethylene levels in plants and promoting plant growth (Ngangom *et al*. 2019; Muniraj *et al*. 2021a, 2021b). Following the planting of winter wheat and spring barley, the addition of *B. megaterium*, *Trichoderma reesei*, and *Acinetobacter calcoaceticus* to the soil led to increased total soil organic carbon content, soil carbon-to-nitrogen ratio, and HA content, and also enhanced microbial diversity (Jurys & Feizienė 2021).

Additionally, the supplementation of *B. subtilis* in composts composed of cow dung and rice straw boosts the levels of total organic carbon and HS, facilitates the humification of dissolved organic matter (DOM) in rice straw

composts, and enriches microbial community diversity within the compost (Duan *et al*. 2020; Qu *et al*. 2024).

This study aims to investigate the gut microbiota of beetles and identify strains capable of converting plant substrates into HA, thereby facilitating the decomposition of agricultural waste. By locally decomposing agricultural residues such as straw into HS, soil fertility can be improved, chemical fertilizer application reduced, soil carbon sequestration enhanced, and agricultural carbon emissions mitigated.

MATERIALS AND METHODS

Collection and taxonomic identification of beetle larvae

Three species of beetle larvae were collected from discarded wood piles in Wufeng District, Taichung City, grain warehouse in Toucheng Township, Yilan County, and decaying jackfruit fruits in Citong Township, Yunlin County. Due to the difficulty of identifying insect larvae based on external morphology, the mitochondrial cytochrome oxidase subunit 1 (COI) gene was amplified using polymerase chain reaction (PCR) and sequenced for species identification using DNA barcoding. First, larval epidermal tissue was placed in a 1.5 mL microcentrifuge tube, and 300 μL of TNES buffer containing 0.1 μg of Proteinase K (50 mM Tris, pH 7.5, 400 mM NaCl, 20 mM ethylenediaminetetraacetic acid (EDTA), 0.5% sodium dodecyl sulfate (SDS)) was added. The reaction was carried out at 37℃ for 3−18 h. Subsequently, 85 μL of 5 M NaCl was added, mixed with a Vortex (Vortex-Genie® 2; Scientific Industries, NY, USA) for 15 s, and centrifuged at 14,000 g for 5 min. The supernatant was collected and mixed with an equal volume of 99% ethanol, followed by centrifugation to precipitate DNA. Finally, the DNA pellet was washed with 70% ethanol, centrifuged again, and resuspended in 20 μL of ddH_2O (Sunnucks & Hales 1996). PCR was conducted using the Takara Taq™ kit (R001B, Takara Bio USA Inc., San Jose, CA, USA) in a reaction volume of 50 μL. Each reaction included 5 μL of the aforementioned DNA solution and 0.5 μL of a primer mix consisting of 20 mM each of the following primers: LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al*. 1994), along with 0.5 μL of Taq polymerase, 5 μL of 10× buffer, 4 μL of dNTPs, and 34.5 μL of ddH_2O. Amplification was carried out using a thermal cycler (GeneAmp® PCR System 2400, Perkin-ElmerInc., Shelton, CT, USA). The PCR conditions consisted of an initial denaturation step at 94℃ for 5 min, followed by 35 cycles of 94℃ for 30 s, 50℃ for 1 min, 72℃ for 1 min, and a final extension at 72℃ for 7 min. The PCR products were sequenced by Genomics Inc. (New Taipei, Taiwan) using an ABI 3730XL DNA Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The obtained DNA sequences were compared to the COI gene sequences in the GenBank database using the Basic Local Alignment Search Tool (BLAST) (National Center for Biotechnology Information (NCBI), Bethesda, MD, USA) to identify the beetle species.

Gut microbiota analysis of 3 beetle larvae

Three larvae from each of the three beetle species were dissected to isolate their gut tissues. The gut tissues were then sent to Genomics Inc. (New Taipei, Taiwan) for metagenomic analysis. PCR amplification of the V3−V4 region of the 16S rRNA gene was performed using the 341F and 805R primers. The PCR products were purified using the AMPure XP system (Beckman Coulter, Brea, CA, USA) and used to construct libraries with the Nextera XT Index Kit v2 Set (Illumina Inc., San Diego, CA, USA). Library quality was monitored using the Qubit 2.0 and Qsep400 System (Bioptic Inc., New Taipei, Taiwan). Finally, sequencing of 300 bp paired-end reads was conducted using the Illumina MiSeq system (Illumina Inc., San Diego, CA, USA).

Isolation and identification of gut bacteria in beetle larvae

The larvae of the 3 beetle species were surface-sterilized with 70% ethanol. Subsequently, their gut tissues were dissected using scalpels and forceps and placed in lysogeny broth (LB). The LB was then serially diluted tenfold and spread onto nutrient agar (NA) and LB agar plates. After incubation at 30℃ for 72 h, individual bacterial colonies were transferred into 1.5 mL microcentrifuge tubes containing 10 μL of 1 mM Tris-hydrochloric acid (HCl) buffer. The tubes were subjected to a heat-cold treatment: 5 min in ice, followed by 1 min at 95℃, 30 s in ice, repeated for a total of 4 cycles, and a final 5-min ice bath to lyse the bacterial cells and release DNA into the Tris-HCl buffer.

PCR was performed using the Takara Taq™ kit (R001B, Takara Bio USA Inc., San Jose, CA, USA) with a reaction volume of 50 μL. Each reaction contained 1 μL of the aforementioned DNA solution, 0.5 μL of a primer mix consisting of 20 mM each of the following primer pairs: 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-TACCTTGTTACGACTT-3'), or 338F (5'-ACTCCTACGGGAGGCAGCAG-3') and 806R (5'-GGACTACHVGGGTWTCTAAT-3') (Frank *et al.* 2008; Armingohar *et al.* 2014), along with 0.5 μL of Taq polymerase, 5 μL of 10× Buffer, 4 μL of dNTPs, and 34.5 μL of ddH_2O. PCR amplification was carried out using a thermal cycler (GeneAmp® PCR System 2400, PerkinElmer, Inc., Shelton, CT, USA). The PCR conditions consisted of an initial denaturation step at 94℃ for 10 min, followed by 35 cycles of 94℃ for 30 s, 55℃ for 30 s, and 72℃ for 1 min, with a final extension step at 72℃ for 7 min to amplify the 16S rRNA gene sequence. The PCR products were sequenced by Genomics Inc. (New Taipei, Taiwan) using an ABI 3730XL DNA Analyzer (Thermo Fisher Scientific Inc., Waltham, MA, USA). The obtained DNA sequences were compared to the 16S rRNA gene sequences in the GenBank database using the BLAST (NCBI, Bethesda, MD, USA) to confirm gut bacteria.

Bacteria selection for HA synthesis

Gut bacteria isolated from the 3 beetle larvae were tested for cellulase, ligninase, laccase, and tyrosinase activities. Cellulase activity was assessed using 0.1% (w/v) carboxymethyl cellulose (CMC)-LB agar plates, followed by Congo red staining (Gohel *et al.* 2014). Ligninase activity was measured in a medium containing 2.0 g $(NH_4)_2SO_4$, 0.5 g $MgSO_4$, 1.0 g K_2HPO_4, 0.5 g NaCl, and 5.0 g alkaline lignin, supplemented with 1% (w/v) aniline blue (Xiong *et al.* 2020). Laccase activity was determined using a medium containing 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Ahmed *et al.* 2018), while tyrosinase activity was screened in a medium supplemented with 0.1% L-tyrosine (Valipour & Arikan 2015).

Evaluation of bacterial conversion of rice straw into HA

Detection of strains with HA biosynthetic enzymes for their HA conversion efficiency on rice straw was measured at 14, 28, and 42 d. Three Erlenmeyer flasks were prepared with 10 g of ground dry rice straw each, sterilized at 120℃ for 20 min. Each flask was inoculated with 1 mL of bacterial culture and incubated at 35℃ while shaken at 200 rpm (LM-570R, Yih Der, Taipei, Taiwan) for 2 d, followed by the addition of sterile water to achieve 65−70% moisture content. Humification was carried out at 30℃, while the control group received only 1 mL of LB. HA content was measured every 14 d from one flask for each treatment, with 3 replicates per treatment. The HA detection method followed the HA inspection procedure outlined in the Taiwan Agricultural Fertilizer Management Standard (AFS2102-1, announced under agricultural food supply letter No. 1091068958A on April 24, 2020).

Rice straw treated with the bacteria was dried at 105℃ for 24 h, and 0.5 g of the dried rice straw powder was placed into each of 8 centrifuge tubes. To each tube, 25 mL of 0.05% sodium lauryl sulfate solution containing 4% HCl was added, shaken at 120 rpm for 1 h,

centrifuged at 2,990 g for 20 min, and the supernatant was removed. This process was repeated with 25 mL of 0.0005% sodium lauryl sulfate solution containing 0.04% HCl, shaken vigorously for 1 min, centrifuged at 2,990 g for 20 min, and repeated once. Subsequently, 25 mL of 0.25 N sodium hydroxide (NaOH) solution was added to each tube, shaken at 120 rpm for 1 h, centrifuged at 7,656 g for 20 min, and the supernatant was transferred to a 100 mL beaker. This step was repeated twice. The combined supernatant was adjusted to a pH ≤ 1 with 6 N HCl, allowed to stand for 10 min, centrifuged at 7,656 g for 20 min, and the supernatant was removed. Subsequently, 25 mL of distilled water was added, shaken vigorously for 1 min, centrifuged at 2,990 g for 20 min, and the supernatant was removed, repeated twice. The precipitate from the 8 tubes was combined, washed into a crucible with distilled water, dried at 105℃ for 24 h, cooled to room temperature, and weighed (W1). The crucible with the precipitate was then placed in a high-temperature furnace ash, heated gradually to 600℃, maintained at this temperature for 4 h, cooled to 100℃, cooled to room temperature in a desiccator, and weighed again (W2).

The conversion efficiency of the bacteria in converting rice straw to HA was calculated using the formula: conversion efficiency (%) = (W1 – W2) / W × 100.

RESULTS

Identification of beetle larvae

Three beetle larvae used in this study were collected from discarded wood piles in Wufeng District, Taichung City, grain warehouse in Toucheng Township, Yilan County, and decaying jackfruit fruit in Citong Township, Yunlin County. Mitochondrial COI gene fragments of 600, 632, and 643 bp were amplified and sequenced using LCO1490/HCO2198 primers (Appendix). The sequences showed 99.3%, 100.0%, and 98.9% similarity to that of *Trypoxylus dichotomus* (GenBank LC074686), *Alphitobius diaperinus* (GenBank MT610905), and *Araecerus fasciculatus* (GenBank KM446808) in the GenBank database, respectively. Therefore, these 3 beetle species were identified as *Tr. dichotomus*, *Al. diaperinus*, and *Ar. fasciculatus*.

Gut microbiota analysis of 3 beetle larvae

The gut microbiota of 3 beetle larvae were analyzed using the V3−V4 region of the 16S rRNA gene. Significant differences were observed in their gut microbiota, with only 1 bacterium, *Corynebacterium* sp., found across all species. *Tr. dichotomus* had a greater diversity and abundance of bacteria compared to the other 2 beetles (see Figs. 1–4). The dominant bacterial families differed among the 3 species: *Tr. dichotomus* had a balanced presence of Ruminococcaceae and Lachnospiraceae, each comprising about 20% of its gut microbiota; *Al. diaperinus* larvae were dominated by Streptococcaceae (around 59%); while *Ar. fasciculatus* larvae from grain warehouse and decayed Polomiti fruits had a simpler gut microbiota, dominated by Lactobacillaceae (approximately 58%).

Isolation and identification of gut bacteria in beetle larvae

We isolated and identified 118 strains of symbiotic bacteria from the intestines of larvae of 3 beetle species: *Tr. dichotomus*, *Al. diaperinus*, and *Ar. fasciculatus*, with 72, 27, and 19 strains isolated, respectively (Table 1). Among the 118 strains of bacteria, the most abundant genus was *Bacillus*, with 34 strains, of which 31 were isolated from the gut of *Tr. dichotomus* larvae. This was followed by *Staphylococcus* with 29 strains, *Citrobacter* with 20 strains, and *Acinetobacter* with 12 strains; all other genera had fewer than 10 strains.

Bacteria selection for HA synthesis

We used selective culture media to assess the cellulase, ligninase, laccase, and tyrosinase activities of 118 bacterial strains isolated from

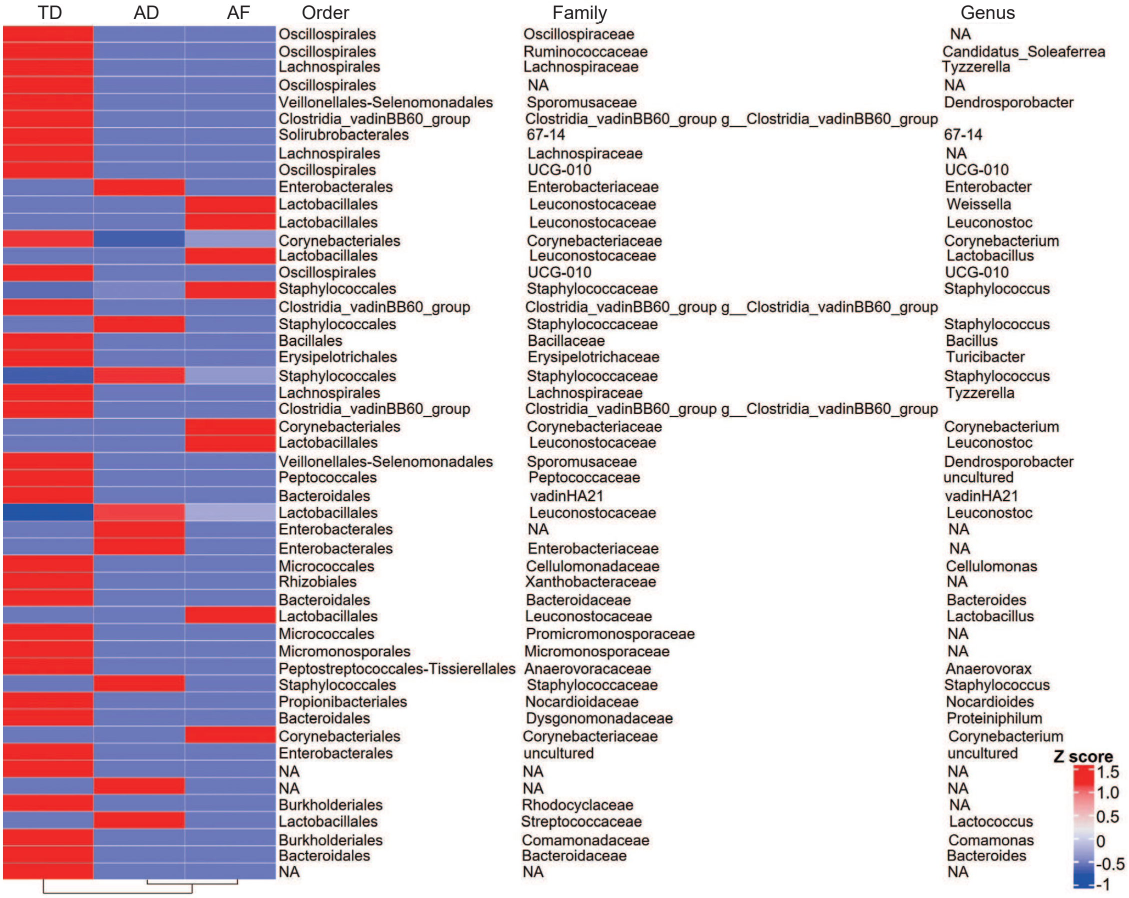

Fig. 1. Heat map of the top 50 abundance microbes in the gut of 3 beetle larvae *Trypoxylus dichotomus* (TD), *Alphitobius diaperinus* (AD), and *Araecerus fasciculatus* (AF).

larvae of 3 beetle species. Among these, 12 strains from *Tr. dichotomus* larvae displayed these enzyme activities. Specifically, *B. aryabhattai* BA01 and *B. megaterium* BM01 exhibited all 4 enzyme activities; *B. subtilis* BS01 showed the first three, while *B. cereus* BC01 and *Neobacillus ginsengisoli* N01 had 2 enzyme activities (Table 2). In contrast, bacteria from *Al. diaperinus* and *Ar. fasciculatus* larvae only showed cellulase activity (Tables 3–4).

Evaluation of bacterial conversion of rice straw into HA

We selected 5 bacterial strains known for their synthesis of HA-related enzymes: *B. subtilis* BS01, *B. megaterium* BM01, *B. aryabhattai* BA01, *B. cereus* BC01, and *N. ginsengisoli* N01, to assess their ability to convert rice straw into HA. After treating the rice straw for 14, 28, and 42 d, all 5 strains demonstrated higher HA conversion efficiencies compared to the control group without bacteria. At 14 d, *B. megaterium* BM01 showed the highest conversion efficiency, increasing by 2.2% compared to the non-inoculated control, followed by *B. subtilis* BS01 at 2.1%, with the remaining 3 strains below 2.0%. At 28 d, *B. subtilis* BS01 led with a 2.3% increase, followed by *B. megaterium* BM01 and *B. aryabhattai* BA01 at 2.2% and 2.1%, respectively, with the other 2 strains below 2.0%. By 42 d, *B. subtilis* BS01 again showed the highest effi-

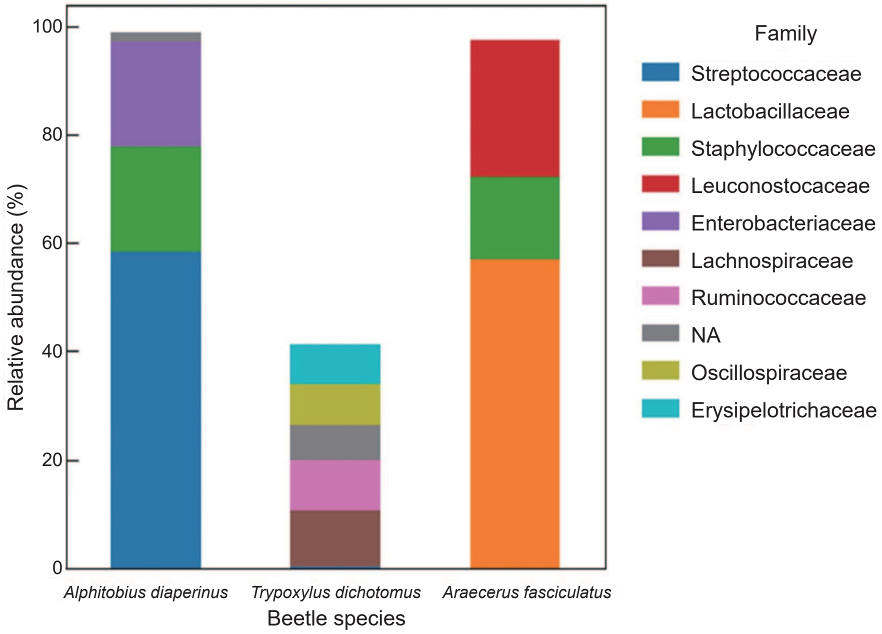

Fig. 2. Relative abundances of bacterial phyla at the family level (top 10) present in the gut of 3 beetle larvae.

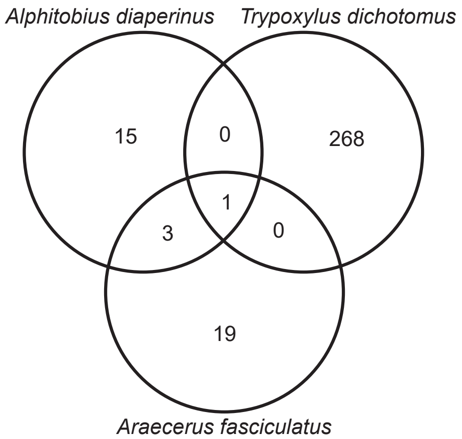

Fig. 3. Venn diagram shows the intersection of the species level in the gut of 3 beetle larvae.

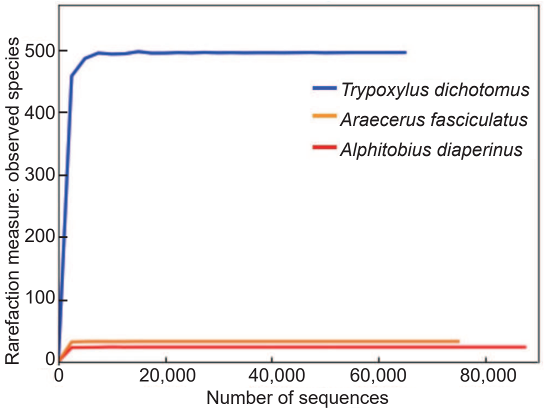

Fig. 4. Rarefaction curves illustrating the species richness of gut microbiota in 3 beetle larvae across different sequencing depths of the 16S rRNA gene.

ciency with a 2.4% increase, followed by *B. aryabhattai* BA01 and *B. megaterium* BM01 at 2.3% and 2.2%, respectively, with the other 2 strains below 2.0%. This indicates that *B. subtilis* BS01, *B. aryabhattai* BA01, and *B. megaterium* BM01 have the highest potential for accelerating the humification of rice straw (Fig. 5).

DISCUSSION

This study examined the gut microbiota of larvae from 3 beetle species with distinct diets. The gut microbiota of *Tr. dichotomus* larvae were found to be richer compared to those of *Al. diaperinus* and *Ar. fasciculatus* larvae. This difference may be due to *Tr. dichotomus* larvae

Table 1. Symbiotic bacteria and their quantities extracted from the guts of 3 beetle species.

Beetle	Species	Numbers	Total numbers
Trypoxylus dichotomus	*Acinetobacter* sp.	12	72
	Bacillus sp.	31	
	Chryseobacterium sp.	1	
	Citrobacter sp.	20	
	Lysinibacillus sp.	2	
	Kluyvera sp.	1	
	Neobacillus sp.	1	
	Streptomyces sp.	1	
	Staphylococcus sp.	3	
Alphitobius diaperinus	*Enterobacter* sp.	8	27
	Pseudomonas sp.	7	
	Sta. sp.	12	
Araecerus fasciculatus	*Bacillus* sp.	3	19
	Paenisporosarcina sp.	1	
	Sta. sp.	14	
	Sporosarcina sp.	1	

Table 2. The enzymatic activity of gut symbiotic bacteria from *Trypoxylus dichotomus*.

Species	Cellulase	Ligninase	Laccase	Tyrosinase
Acinetobacter baumannii A01	+[z]	+	-[y]	-
Bacillus sp.	+	-	-	-
B. subtilis BS01	+	+	+	-
B. megaterium BM01	+	+	+	+
B. aryabhattai BA01	+	+	+	+
B. cereus BC01	+	-	+	-
Chryseobacterium sp.	+	-	-	-
Citrobacter koseri	+	-	-	-
Lysinibacillus xylanilyticus	+	-	-	-
Kluyvera sp.	+	-	-	-
Neobacillus ginsengisoli N01	+	-	-	+
Streptomyces sp.	+	-	-	-

[z] +: Enzyme activity was detected.
[y] -: No enzyme activity was detected.

living in decaying woodpiles in natural soil habitats, which harbor a richer microbial community. When feeding on the decaying substrates within these woodpiles, various microbes are ingested and retained in the gut. Subsequently, we screened the gut microbiota of *Tr. dichotomus* larvae and identified 3 microbial strains, *B. megaterium* BM01, *B. aryabhattai* BA01, and *B. subtilis* BS01, which exhibited enzymes related to HA synthesis. The 3 strains showed the highest potential for accelerating the humification of rice straw. In the future, these strains could assist in on-site decomposition of leftover rice straw after harvest, converting it into HA. This could

Table 3. The enzymatic activity of gut symbiotic bacteria from *Alphitobius diaperinus*.

Species	Cellulase	Ligninase	Laccase	Tyrosinase
Enterobacter sp.	+[z]	-[y]	-	-
E. cloacae Bc01	+	-	-	-
Pseudomonas taiwanensis Bt01	+	-	-	-
Staphylococcus kloosii Bk01	+	-	-	-
Sta. edaphicus Be01	+	-	-	-

[z] +: Enzyme activity was detected.
[y] -: No enzyme activity was detected.

Table 4. The enzymatic activity of gut symbiotic bacteria from *Araecerus fasciculatus*.

Species	Cellulase	Ligninase	Laccase	Tyrosinase
Bacillus anthracis Ba01	+[z]	-[y]	-	-
B. licheniformis Bl01	+	-	-	-
Paenisporosarcina sp.	+	-	-	-
Staphylococcus sp.	+	-	-	-
Sporosarcina sp.	+	-	-	-

[z] +: Enzyme activity was detected.
[y] -: No enzyme activity was detected.

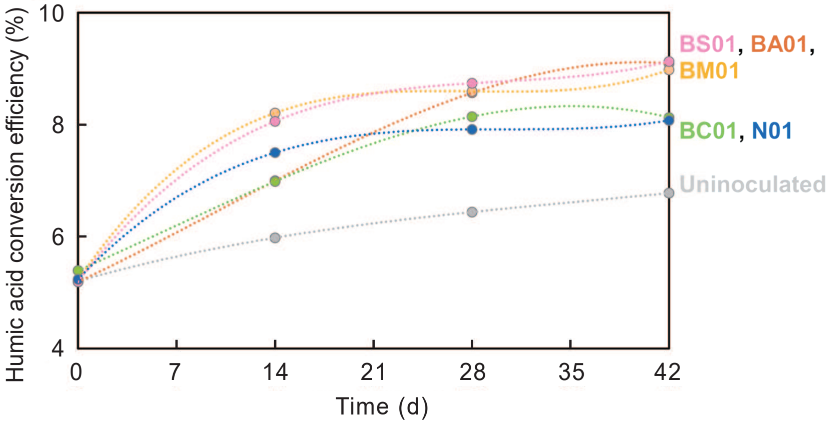

Fig. 5. The convert efficacy of humic acid from rice straw by *Bacillus subtilis* BS01, *B. megaterium* BM01, *B. aryabhattai* BA01, *B. cereus* BC01, and *Neobacillus ginsengisoli* N01.

enhance soil fertility and promote soil carbon sequestration.

The gut microbiota of insects are closely related to their dietary habits. In this study, we examined the gut microbiota of *Al. diaperinus* larvae obtained from grain warehouse and *Ar. fasciculatus* larvae found in decaying jackfruit fruit. Due to their simpler diets, these larvae exhibited less diverse gut microbiota. In contrast, *Tr. dichotomus* larvae, which inhabit environments with more complex food sources, showed a more diverse gut microbiota. The dominant bacterial families identified were Ruminococcaceae and Lachnospiraceae of the phylum Bacillota (formerly Firmicutes). This finding aligns with the research by Huang *et al.* (2022) on scarab larvae, where Bacillota was the most abundant phylum in *Tr. dichotomus*

larvae guts, associated with polysaccharide fermentation.

Additionally, the differences in insect gut microbiota are closely associated with the composition and sources of their diets (Huang *et al*. 2022; Mannaa *et al*. 2023). The richer gut microbiota observed in *Tr. dichotomus* compared to *Al. diaperinus* and *Ar. fasciculatus* larvae may stem from the decaying substrates or surrounding soil in their habitats. Previous research has shown that the cetoniid beetle larvae *Pachnoda ephippiata* selectively utilize HA peptides and polysaccharides from soil substrates and produce higher levels of HA in their feces, suggesting a connection with their gut microbiota (Li & Brune 2005).

The gut microbiota of *Oryctes rhinoceros* larvae, a species belonging to the Scarabaeidae family, have been found to contain bacteria capable of degrading hemicellulose and cellulose, predominantly belonging to the *Bacillus* genus (Sari *et al*. 2016). Similarly, our study identified 12 bacterial strains with cellulose-degrading capabilities in the gut of *Tr. dichotomus* larvae, a species also belonging to the Scarabaeidae family. This suggests that Scarabaeidae beetles have the potential to degrade plant residues through their gut microbiota.

Microbial redox enzymes such as tyrosinase and laccase play a role in organic matter degradation and humification (Binner *et al*. 2011). These enzymes are crucial in the synthesis of HS as they can degrade lignin-like polymers in organic matter and catalyze the cross-linking of amino acids, promoting the formation of humic polymer skeletal structures and ultimately aiding in carbon sequestration (Zavarzina *et al*. 2011).

Several microorganisms, including *Str.* sp., *Azospirillum lipoferum*, *B. aryabhattai*, *B. megaterium*, *Rhizobium* sp., *Thermomicrobium roseum*, and *Vibrio tyrosinaticus*, have been reported to exhibit tyrosinase activity (Pomerantz & Murthy 1974; Kong *et al*. 2000; Claus & Decker 2006; Piñero *et al*. 2007; Fairhead & Thöny-Meyer 2012; Kanteev *et al*. 2013; Guo *et al*. 2015). In our study, both *B. aryabhattai* BA01 and *B. megaterium* BM01 showed tyrosinase activity, and the tyrosinase activity of *N. ginsengisoli* N01 was reported for the first time.

Bacillus species are known for their ability to degrade lignocellulose and are often used in straw composting studies. For instance, inoculating straw with *B. siamensis* (H1), *B. halophilus* (H2), and *B. parahemolyticus* (S1) has been shown to increase the HA content (Zhao *et al*. 2024). In our study, we isolated 3 *Bacillus* strains with the highest HA conversion capabilities from the gut of *Tr. dichotomus* larvae: *B. megaterium* BM01, *B. aryabhattai* BA01, and *B. subtilis* BS01. Muniraj *et al*. (2021a, 2021b) found that *B. aryabhattai* TG5 exhibits laccase and tyrosinase activities. Treatment with its tyrosinase resulted in the formation of HS from coir pith within 3 d, confirming its role in HS formation. Moreover, *B. megaterium* AS019 has been shown to convert vinasse (sugarcane mill wastewater) into HA, helping to reduce pollution from vinasse disposal (Li *et al*. 2018).

Laccase enzymes are typically found in higher plants and fungi but have recently been discovered in some bacteria as well (Shraddha *et al*. 2011) such as *Str. cyaneus* (Arias *et al*. 2003), *Monocillium indicum* (Thakker *et al*. 1992), and *Marinomonas mediterranea* (Jimenez-Juarez *et al*. 2005). In this study, laccase activity was also observed in *B. subtilis* BS01, *B. megaterium* BM01, *B. aryabhattai* BA01, and *B. cereus* BC01.

Moreover, these 3 bacterial strains have been the focus of various agricultural studies. *B. subtilis* can act as a plant growth-promoting agent, aiding in soil phosphorus solubilization, enhancing nitrogen fixation, and producing siderophores to facilitate plant root growth (Hashem *et al*. 2019). Recent findings have shown that *B. subtilis* exhibits antagonistic effects against various pathogens, such as *Phytophthora capsici*, *Blumeria graminis* f. sp. *tritici*, and *Fusarium oxysporum* f. sp. *cucumerinum*, making it one of the most promising microbes for sustainable development (Lin *et al*. 2010; Cao *et al*. 2011; Xie *et al*. 2021).

B. megaterium has been found to suppress

the damage caused by rice root-knot nematode (*Meloidogyne graminicola*) and rice sheath blight (*Rhizoctonia solani*). Even after being formulated into granular preparations and stored for 2 years, it still demonstrated effective control against rice sheath blight (Padgham & Sikora 2007; Chumthong *et al*. 2008). In terms of promoting plant growth, *B*. *megaterium* possesses the ability to synthesize auxins and cytokinins and easily aggregates in the rhizosphere and soil, promoting plant growth under stress conditions (Nascimento *et al*. 2020).

B. *aryabhattai* is a phosphate-solubilizing bacterium that converts phosphate into a plant-available form, enhances nitrogen uptake, promotes plant growth, and increases crop yields (Ramesh *et al*. 2014; Wu *et al*. 2019). These findings broaden the potential applications for the 3 selected strains in this study, *B*. *megaterium* BM01, *B*. *aryabhattai* BA01, and *B*. *subtilis* BS01, particularly in enhancing carbon sequestration capabilities.

CONCLUSION

We explored the gut microbiota of 3 beetle species with different diets, confirming that diet influences the gut microbiota composition. With the aim of recycling agricultural waste and enhancing soil carbon sequestration, we screened for bacterial strains capable of transforming HA and verified their ability to convert straw into HA. Among them, *B*. *subtilis* BS01, *B*. *aryabhattai* BA01, and *B*. *megaterium* BM01 exhibited the highest potential for straw humification. These strains can accelerate the decomposition of agricultural waste in the field, facilitating the return of HS to the soil, increasing the residence time and stability of organic carbon in the soil, and enhancing soil carbon sequestration to promote carbon-negative farming practices. Furthermore, both *B*. *subtilis* and *B*. *megaterium* have been reported to suppress plant diseases, while *B*. *aryabhattai* has been found to promote crop growth; therefore, further expansion of the potential applications of these 3 bacterial strains is warranted in the future.

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Appendix. Partial mitochondrial cytochrome oxidase subunit 1 (COI) gene sequences of the three beetle larvae.

Trypoxylus dichotomus
CAGAATTAGGGAATCCAGGCTCTCTGATTGGAGATGATCAAATTTATAATGTAATTGTAACTGCCCATGCTTTTAT-
TATAATTTTCTTTATAGTAATACCTATTATAATTGGAGGATTTGGTAATTGACTTGTGCCTTTAATACTTGGGGCTC-
CAGATATAGCATTTCCCCGAATAAATAATATAAGATTTTGACTTTTACCCCCTTCTTTAACTCTTTTATTAG-
CAAGAAGAATAGTAGAAAATGGGGCTGGAACAGGATGAACAGTTTACCCTCCACTATCTGCTAATATTGCTCATAGTG-
GAGCATCTGTTGATTTAGCTATTTTTAGTCTTCATCTAGCAGGAATTTCATCTATTTTAGGGGCTGTAAATTTTATTACAA-
CAGTAATTAATATACGATCAACAGGCATAACTTTTGACCGAATACCTCTATTTGTATGATCAGTTATACTAACAGCCATCT-
TACTCCTTCTTTCATTGCCTGTATTAGCAGGGGCTATTACTATACTTCTTACTGACCGAAATATTAATACTTCATTTTTT-
GACCCTGCAGGTGGAGGAGACCCAATTCTATACCAACATCTCTTTTGATTTTTTG

Alphitobius diaperinus
TTGGTACTTCTCTAAGACTACTTATTCGAGCTGAACTTGGAAATCCTGGATCACTAATTGGGGATGACCAAATTTATA-
ACGTTATTGTTACAGCTCACGCATTCATTATAATTTTCTTTATAGTAATACCAATTATAATTGGAGGGTTTGGAAACT-
GACTTGTTCCCCTTATACTTGGTGCCCCAGACATAGCATTCCCTCGAATAAACAATATAAGATTCTGACTTCTC-
CCACCCTCTTTAACCCTTCTTCTAATAAGAAGAATTGTAGAAAGAGGAGCAGGAACTGGATGAACAGTGTAC-
CCCCCACTCTCTTCCAATATTGCACACGGAGGGTCTTCTGTAGACCTAGCCATTTTCAGACTCCATTTAGCTGG-
TATTTCATCAATCCTCGGAGCTGTAAATTTTATCACAACAGTAATTAATATACGACCACAAGGAATAACTTTTGATC-
GAATACCTTTATTTGTATGAGCTGTCGTAATTACAGCAGTTCTTCTTCTTCTTTCACTCCCTGTTCTAGCAGGAGCAAT-
TACAATATTACTTACAGACCGAAATATTAATACCTCATTCTTTGACCCAGCTGGGGGAGGAGACCCAATTTTATATCAA-
CACTTATTTTGATTTTTTTGG

Araecerus fasciculatus
TTGAGCAGGAATAATAGGAACATCCTTAAGAATCTTAATTCGAACAGAATTAGGGAATCCCGGGTCTCTAATTGGAGAT-
GACCAAATCTATAACGTAATCGTAACTGCCCACGCATTCGTTATAATCTTTTTTATAGTAATGCCGACTATAATTGGAG-
GATTTGGGAATTGATTAGTTCCGTTAATACTTGGTGCTCCTGATATAGCATTCCCTCGACTTAACAATATGAGATTTT-
GATTGCTGCCCCCTTCCTTGTCTCTATTGACCTTAAGAAGAATCGTTGAAAGAGGTGCGGGGACTGGTTGAA-
CAGTTTACCCCCCACTATCAGCAAATATTGCCCATAATGGATCTTCTGTTGACTTGGCTATCTTTAGTCTTCATTTAGCAG-
GGGTATCTTCTATTTTAGGAGCAGTTAACTTTATTACTACGATTATCAACATGCGTCCGAGAGGTATATCCCCTGATC-
GTTTACCCCTCTTTGTCTGATCTGTCGGAATCACCGCCCTTCTTCTTTTATTATCTTTACCTGTACTTGCTGGAGCAAT-
TACGATACTCTTAACTGATCGAAATCTAAACACTTCTTTTTTTGACCCCGCAGGAGGAGGTGATCCAATTCTCTACCAA-
CATTTATTTTGATTTTTT

三種甲蟲幼蟲腸道菌相與菌種轉化腐植酸潛力

王泰權 [1] 葉千榕 [2] 林祐丞 [2] 林柏文 [3] 姚美吉 [4] 張淑貞 [4,*]

摘要

王泰權、葉千榕、林祐丞、林柏文、姚美吉、張淑貞。2024。三種甲蟲幼蟲腸道菌相與菌種轉化腐植酸潛力。台灣農業研究 73(3):181−196。

隨著全球氣溫攀升，移除大氣中的 CO_2 並長期封存已是全球的共同目標。農業廢棄物則可藉由微生物轉化為在土壤中不易分解的腐植質 (humic substances; HS)，以達到增加土壤碳匯的目標。本研究分析 3 種不同食性的甲蟲幼蟲腸道微生物相，其中以取食腐朽木材的獨角仙 (*Trypoxylus dichotomus*) 幼蟲腸道菌相較外米擬步行蟲 (*Alphitobius diaperinus*) 及長角象鼻蟲 (*Araecerus fasciculatus*) 的幼蟲腸道菌相較豐富、菌種數量亦較多。進一步分析 3 種甲蟲幼蟲腸道菌的腐植酸生合成相關酵素的活性，包括纖維素酶、木質素酶、漆酶及酪胺酸酶，其中 *Bacillus megaterium* BM01 與 *B. aryabhattai* BA01 可見上述 4 種酵素活性，*B. subtilis* BS01 則有前 3 種酵素活性。進一步檢驗菌株將稻草轉化為腐植酸的能力，其中 *B. megaterium* BM01、*B. aryabhattai* BA01 及 *B. subtilis* BS01，腐植酸轉換效率較未接菌種的對照組，各增加 2.4%、2.3% 及 2.1%。期待未來可應用於稻穀收穫後，田間殘存稻草的現地轉化分解成腐植酸，以達到增加土壤碳匯的目標。

關鍵詞：甲蟲、腸道菌、腐植酸、土壤碳匯。

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椰塊大小對文心蘭生長及切花產量之影響

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摘要

賴思倫、鍾淨惠、戴廷恩。2024。椰塊大小對文心蘭生長及切花產量之影響。台灣農業研究 73(3):197–206。

本研究以不同尺寸椰塊 (50% 0.7−1.0 cm chip + 50% peat 與 1.2−1.5 cm chip) 並搭配自動滴灌系統進行文心蘭「檸檬綠」切花於防雨設施內進行栽培。結果顯示種植 2 mo 內可見新根正常生長，但栽培 20 mo 後，介質開始出現崩解，又以較小尺寸之椰塊崩解速度較快，幾乎已完全變為粉狀。介質物化性分析，pH 值與孔隙度皆顯著下降；植株生長假球莖周徑並無顯著差異，僅於當代假球莖上之第二片葉，以規格 1.2−1.5 cm 之椰塊所栽培的較大；開花品質亦以較大尺寸椰塊之栽培效果較佳，擁有較高之切花產量與品質。整體結果顯示，椰塊可作為文心蘭切花生產用介質，但規格上應選擇較大尺寸，以減緩長期栽培介質崩解所引發之負面影響。

關鍵詞：文心蘭、替代介質、椰塊、切花品質。

前言

文心蘭為臺灣輸日最大宗切花，2023 年外銷產值可達 1,425 萬美元，為重要輸出花卉 (財政部關務署統計資料庫，https://portal.sw.nat.gov.tw/APGA/GA30)。由於文心蘭環境適應力強，容易栽培，全臺幾乎皆可栽培，中南部栽植面積較多，目前栽培面積約 248 ha，主要產區為臺中市、屏東縣以及雲林縣等 (農情報告資源網，https://agr.afa.gov.tw/afa/afa_frame.jsp)。

臺灣文心蘭多以未防雨簡易網室，搭配碎石、木炭或樹皮等排水性強介質，單一或是混合使用進行栽培 (Lai *et al*. 2015)。臺灣地處亞熱帶海洋氣候地區，在國際的氣候變遷研究中屬於高風險的邊緣區，過去資料顯示臺灣氣候已呈現暖化、降雨型態改變、極端氣候發生頻率及強度增加的趨勢，均影響農業生產 (Guo 2011)。面對氣候環境趨向急遽化，文心蘭近年來常有因瞬間大雨造成植株盆傾倒、花梗折損及花瓣受傷的狀況，同時也增加病菌入侵機會，導致切花品質劣化、瓶插壽命減短。進入設施防雨雖是有效因應手段，但碎石介質無法保水保肥的特性，必須每日澆灌，耗費水資源外，更因介質吸附力差，造成肥料隨著頻繁澆灌而流失，無法被有效吸收。考量切花穩質與穩量，提升生產效能與永續發展，必須於設施內進一步改善傳統碎石介質栽培模式。

椰類資材商品規格種類繁多，包含有椰殼粉、椰塊及椰纖等，面對近年來不可再生資材如泥炭土與水苔所引發之產業、社會及生態問題，椰類商品為可再生的副產品與可生物降解的特性已成為替代介質的首選 (Machado *et al*. 2021)。本研究以不同尺寸之椰塊為替代介質，於防雨設施內進行文心蘭切花栽培生產之可行性評估，以提供文心蘭切花防雨設施下栽培模式建立之參考。

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材料與方法

植物材料與介質準備

試驗文心蘭為「檸檬綠」切花 (*Oncidesa* Gower Ramsey 'Honey Angel') (購自臺中市秉薪蘭園)。取原種植於水苔帶 1 假球莖與 1 新芽之植株，假球莖平均周徑約 85 mm，新芽高度平均約 11 cm，保留原包覆水苔，直接使用試驗介質栽種於直徑 12.0 cm 黑色塑膠盆，置於臺中市新社區秉薪蘭園溫室 (經緯度 24°12' 56.5" N 120°46' 41.8" E)，試驗期間環境溫度日/夜溫範圍為 18–28℃/11–23℃，日間光度約 150–200 μmol m^{-2} s^{-1}，中午最高光度約 200 μmol m^{-2} s^{-1}。本試驗栽培介質為小粒椰塊 (50% 0.7–1.0 cm chip + 50% peat) 與大粒椰塊 (1.2–1.5 cm chip) (coir, Riococo, Ceyhinz Link International Inc., Bamunakotuwa, Sri Lanka) (圖 1A)。試驗期間配合滴灌設施進行水分管理，滴灌頻度設定依介質失水狀態，大粒椰塊每週給水 2 次，小粒椰塊每週給水 1 次，每週施用水溶性肥料 0.5 g L^{-1} Peters 20N-8.7P-16.6K (Scotts Co., Marysville, OH, USA) 液肥 1 次。

試驗方法

栽培介質理化特性分析

1. pH 值與電導度值 (electrical conductivity; EC) 分析：依據 Chang *et al*. (2006) 檢測水苔與人工水苔之方法，分別逢機取 3 g 之供試介質，各重複 3 次，加入 100 mL 去離子水 (介質：水 = 1 : 30 (w/v))，浸泡 24 h 後，以手提式酸鹼度計 (pH-meter, TS1, Suntex, New Taipei, Taiwan) 與電導度計 (Conductivity Meter, Sc-12 meter, Suntex, New Taipei, Taiwan) 進行 pH 值與 EC 值測定。
2. 最大保水量與孔隙度分析：參考 Chang *et al*. (2006) 之方法，量秤直徑 7.5 cm，容積 250 mL 之透明塑膠軟盆重 (a)，將浸泡脫水處理後之試驗介質，按慣行栽培之鬆緊度 (裝填高度 7.0 cm，裝填容積 160 mL) 裝填入該透明塑膠軟盆，每種介質處理試驗 6 重複，每重複 1 盆，裝填之後移置通風之烘箱 (forced-draft oven)，以 70℃烘乾 48 h，使其達恆重，並量秤其總重 (b)，(b) − (a) 即為每盆介質乾重，將烘乾後之軟盆與介質移入水浴，靜待水位淹過所有介質後，移出水浴，靜置排水 10 min，重複水浴排水步驟 3 次，量秤其總重 (c)，(c) − (b) − (a) 即為每盆介質最大保水量，[(c) − (b) − (a)] × $[(b) - (a)]^{-1}$ 即為單位重介質最大保水量。將飽水之軟盆與介質移至溫室 (日/夜溫為 23/18℃)，每日量秤軟盆與介質總重 (g)，(g) − (b) − (a) 即為不同時間之介質含水量，再將介質重新烘乾使其達恆重，用有刻度之 1,000 mL 量筒，盛水 500 mL，將烘乾之介質用玻棒壓入水中，振盪 30 min，待介質排出空氣完全吸水後，記錄水位上升刻度 (d)，(d) − 500 mL 即為介質體積，(b) − (a) × $[(d) - 500]^{-1}$ mL 即為介質真比重 (real density)，(b) − (a) × (裝填容積 160 mL) 即為介質容積比重 (bulk density)，而孔隙度 (porosity, %) = 100 × (1 − 介質容積比重 × 介質真比重 $^{-1}$) (Chang *et al*. 2006)。

植株生育調查

1. 營養生長，於種植第 20 個月與第 26 個月 (前後兩代當代假球莖肥大時期) 進行調查。
   (1) 葉片性狀：葉長，取新芽形成假球莖後由上往下數第二葉基部至葉尖長度；葉寬，新芽形成假球莖後由上往下數第二葉最寬處；葉面積，採估算方式，參考 Lin & Lee (1988) 方式，取植株由上往下數第二葉 10 片，量測葉片最大長度與寬度，並以葉面積儀 (LI-300A, LI-COR Inc., Lincoln, NE, USA) 實際量測葉面積，帶入算式求得 K 值為 0.76，得到葉面積估算值 (cm^2) = 長 × 寬 × 0.76，將實際平均葉面積與估算平均葉面積值進行直線回歸，其回歸方程式之係數與決定係數 (R^2) 等在統計分析上皆具存在意義 (圖 2)，表示利用所建立之估算模式估計而得之葉面積估算值與實際葉面積頗為接近，故試驗葉面積皆以該方式估算。
   (2) 假球莖厚度、寬度及周徑，調查新生假球莖之最寬、最厚及最大周徑。

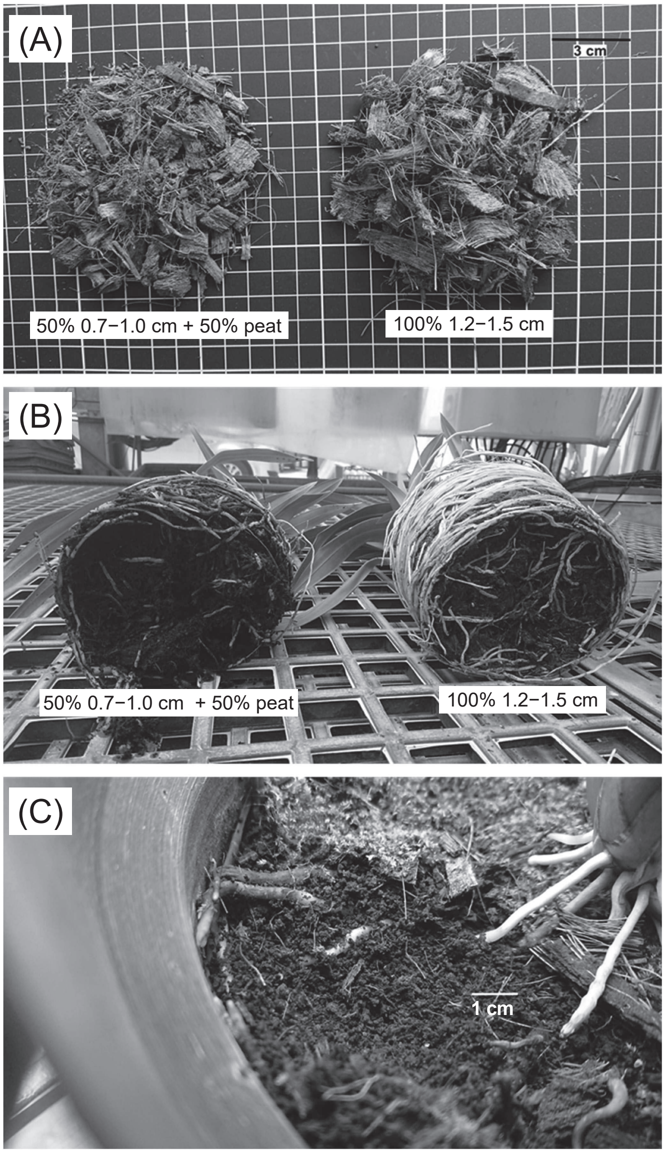

圖 1. (A) 2 種不同尺寸椰塊外觀；(B) 以不同尺寸椰塊種植 26 mo 後根系生育狀況；(C) 種植 26 mo 後小尺寸椰纖崩解狀況。

Fig. 1. (A) The appearance of two different sizes of coir chip; (B) root growth status of coir chip of different sizes after planting for 26 mo; and (c) disintegration status of small-sized coir chip after 26 mo of planting.

2. 開花調查，於種植後的第 2 次開花期開始進行，並依商業分級標準進行每月產量調查開花性狀，由花梗基部測量至花梗末梢長度為花梗長度；自基部往上第一側枝至花梗末梢長度為花序長度；花梗去除未形成花苞等異常側枝之總側枝數為花梗分枝數。

試驗設計與統計分析

試驗設計採完全逢機設計 (complete randomized design; CRD)，每種介質試驗 20 重複，每重複 5 株。試驗數據以 *t* 檢定進行顯著差異分析或以統計軟體 Costat 6.1 (CoHort Software, USA) 進行變方分析與最小顯著差異

性測驗 (Fisher's least significant test, LSD, $P < 0.05$) ，並以 Microsoft Office Excel 2007 進行繪圖。

結果

介質理化性分析

分析結果顯示 (表 1)，種植前後，EC 並無顯著差異。種植前 pH 值以小粒椰塊略高於大粒椰纖，栽培 26 mo 後，兩種介質之 pH 值皆明顯下降，其中又以小粒椰塊下降幅度較高，但統計上並無顯著差異。栽培前後皆以小粒椰塊之保水力較高，栽培 26 mo 後，兩種椰塊之保水能力皆呈現上升的現象。小粒椰塊於種植前孔隙度明顯低於大粒椰塊，長期栽培後孔隙度變化，兩者介質呈現不同趨勢。栽培 26 mo 後，小粒椰塊孔隙度明顯下降，同時目視可見介質出現明顯崩解現象 (圖 1C)，而大粒椰塊之孔隙度反而呈現上升現象。

不同尺寸椰塊對文心蘭生育與切花品質之影響

以大小顆粒之椰塊進行文心蘭栽培，於定植 2 mo 後皆可見新根長出 (圖 3)，種植 20 mo 後，假球莖的尺寸上並無顯著差異，但小粒椰塊種植之文心蘭植株葉片長度顯著較短，相對於大粒椰塊種植植株，葉面積減少約 15.8% (表 2)。種植 26 mo 後，兩處理於生長勢上出現明顯差異，相對於大粒椰塊種植植株，小粒椰塊種植之文心蘭假球莖高度減少約 7%，葉長與葉面積亦分別減少約 8% 與 13.4% (表 2)。另透過目視亦可見小粒椰塊種植植株之根系出

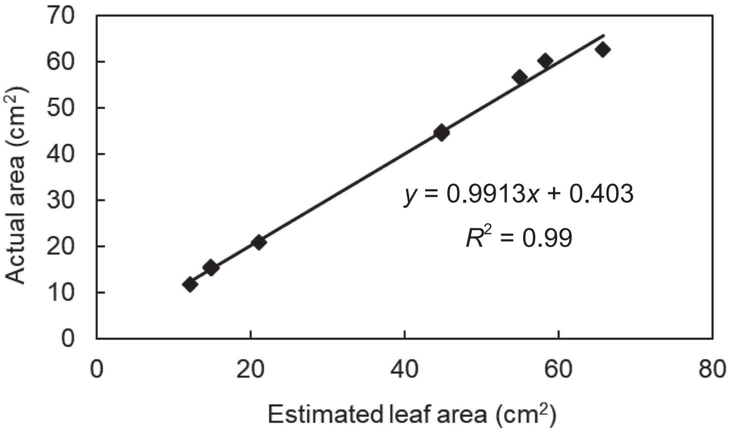

圖 2. 以共同常數估值估算之葉面積與實際葉面積之直線回歸。

Fig. 2. Relationship between actual and pooled estimated leaf area (max. length × max. width × 0.80) for examining the adequacy of the estimated model.

表 1. 栽培介質物理與化學特性。

Table 1. The physical and chemical characteristics of growing medium.

Growing medium	Usage time (mo)	pH	EC[z] (dS m^{-1})	Water holding capacity per pot (g)	Porosity (%)
50% 0.7−1.0 cm chip + 50% peat	0	6.9 a[y]	0.10 a	61.51 b	21.97 c
	26	4.4 c	0.11 a	68.20 a	20.76 d
1.2−1.5 cm chip	0	6.7 b	0.09 a	38.54 d	47.73 b
	26	4.6 c	0.08 a	47.30 c	49.09 a

[z] EC: electrical conductivity.
[y] Mean ± standard error ($n = 5$). Means separation the same column with the same letter are not significantly different at $P < 0.05$ by Fisher's protected least significance difference (LSD) test.

現衰弱甚至死亡現象，以大粒椰塊種植之文心蘭植株根系則豐富且健康 (圖 1B)。

圖 3. 種植 2 mo 後可見新根長出。

Fig. 3. New roots can be seen growing 2 mo after planting.

切花品質，以大粒椰塊種植植株較佳，尤其以花梗與花序長度，以及第一側枝長度之增加最明顯，可分別增加 5.7%、4.6% 以及 13.1% (表 3)。切花產量與等級，種植第 20−24 個月，大粒椰塊種植切花總產量為 140 支，其中可外銷等級 (3−4 L) 支數占總產量 90.7%，而小粒椰塊種植切花總產量為 120 支，可外銷等級 (3−4 L) 支數占總產量 78.3%，大粒椰塊種植生產切花總產量增加約 16.6% (表 4)。切花產期，兩種介質尺寸並無顯著影響，切花盛產期均落在同一時間 (圖 4)。

討論

栽培介質理化性分析

椰塊是椰子中果皮經去除外果皮與果肉之纖維製品，其結構均勻統一，含有鉀、鈣、鈉

表 2. 以不同尺寸椰塊種植文心蘭假球莖與葉片生長勢。

Table 2. Growth potential of pseudobulbs and leaves after planting *Oncidium* with coir chip of different sizes.

		Current pseudobulb		Leaf of current pseudobulb		
Planting time	Growing medium	Circumference (cm)	Height (cm)	Length (mm)	Width (mm)	Area (mm^2)
20 mo	50% 0.7−1.0cm chip + 50% peat	8.84	8.70	35.40	3.36	95.85
	1.2−1.5 cm chip	9.76	9.26	39.61	3.60	113.84
	significant^z	n.s.^{y}	n.s.	*	n.s.	*
26 mo	50% 0.7−1.0cm chip + 50% peat	8.52	8.53	36.15	3.14	92.13
	1.2−1.5 cm chip	9.85	9.18	39.48	3.35	106.44
	significant	*	*	*	n.s.	*

^{z} Two-tailed Student's *t*-test was used to determine the significance of the indicated comparison.
^{y} n.s.: not significant.
* $P < 0.05$.

表 3. 以不同尺寸椰塊種植文心蘭 20−24 mo 之開花品質。

Table 3. Flowering quality of *Oncidium* planted with coir chip of different sizes in 20−24 mo.

Growing medium	Length of flower-stalk (cm)	Length of inflorescence (cm)	No. of side branches (No.)	Length of the first side branches (cm)
50% 0.7−1.0 cm chip + 50% peat^z	121.37	57.91	9.86	20.16
1.2−1.5 cm chip^y	128.24	60.55	10.11	22.80
significant^x	*	*	n.s.^{w}	*

^{z} $n = 120$.
^{y} $n = 140$.
^{x} Two-tailed Student's *t*-test was used to determine the significance of the indicated comparison.
^{w} n.s.: not significant.
* $P < 0.05$.

表 4. 以不同尺寸椰塊種植文心蘭 20–24 mo 之切花產量與等級。

Table 4. Cut flower yield and grade of *Oncidium* planted with coir chip of different sizes in 20–24 mo.

Growing medium	Total production (No.)	Grade[z]			
		4 L (No.)	3 L (No.)	2 L (No.)	L (No.)
50% 0.7–1.0 cm chip + 50% peat	120	66	28	22	4
1.2–1.5 cm chip	140	114	13	7	6

[z] Grading standards. L: Length of flower-stalk ≥ 60 cm, No. of side branches ≥ 2, Length of the first side branches ≥ 10 cm; 2 L: Length of flower-stalk ≥ 70 cm, No. of side branches 3–4, Length of the first side branches ≥ 10 cm; 3 L: Length of flower-stalk ≥ 80 cm, No. of side branches 5–6, Length of the first side branches ≥ 15 cm; 4 L: Length of flower-stalk ≥ 90 cm, No. of side branches ≥ 7, Length of the first side branches ≥ 20 cm.

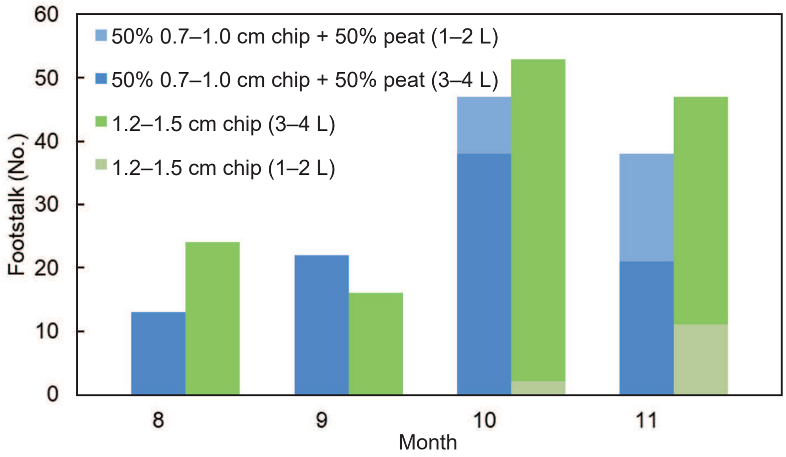

圖 4. 以不同尺寸椰塊種植 20–24 mo 每月切花產量與等級變化。

Fig. 4. Changes in monthly cut flower yield and grade after planting coir chip of different sizes for 20–24 mo.

及鎂等植物生長必須元素 (Dong *et al*. 2011)。過去的研究，亦有多篇研究將椰類製品應用於作物種植上，Wang *et al*. (2015) 比較不同栽培介質對文心蘭出瓶苗生長之影響，結果顯示以椰糠、泥炭與河沙混合介質以及椰纖介質之地上部生長勢最佳，而椰纖由於取得方便，成本低且前處理較省時省力，更適合作為文心蘭栽培推廣使用。將不同尺寸椰纖混合後，可維持較高的保肥與緩衝能力，可作為火鶴切花生產系統之栽培介質 (Chang *et al*. 2011)。

介質常為植物生長所必須，具有貯存養分、保持水分、通氣良好及固定植物之作用 (Lee 1989)。當 EC 值大於 2.0 dS m^{-1} 時，介質所含之鹽類量即會影響作物之生長 (Chang 1987)。以介質與水以 1 : 5 (w/v) 比例進行電導度測量，發現當 EC 值高於 2.33 dS m^{-1} 以上時，導致根部養分吸收發生障礙，植體乾重與根乾重均呈明顯的降低現象 (Lo & Wang 2000)。介質電導度過高表示可溶性總鹽類太多，植物鹽害發生的機率也會上升，通常介質飽和抽出液之 EC 高過 2.25 dS m^{-1}，便會造成植物萎凋 (Wang *et al*. 2006)。本試驗雖非使用飽和抽出液做測量 (介質：水 = 1 : 30 (w/v))，但檢測栽培試驗介質之 EC，無論栽培時間長短皆介於 0.08–0.11 dS m^{-1} 之間，符合一般介質理想標準 (表 1)。pH 值為介質酸鹼度，影響植株對於養分吸收的能力。pH 值一般建議應該在 5.4–6.0 之間，可使大部分的礦物元素有效溶解被植物吸收 (Shen 2007)。介質過酸將導致鋁、鐵、鋅、銅及錳等礦物元素的釋出溶解度加快，這可能會對植物產生毒害風險 (Yen 2013)。然實際 pH 值標準仍需視作物特性而定，文心蘭小苗對 pH 值的可接受範圍約為 3–6 (Tsai 2000)。本試驗兩種介質於種植前

的 pH 值雖超過 6，但隨使用時間的拉長會逐漸下降至 4.4 與 4.6 (表 1)，試驗調查期間皆在 pH 值的容許範圍。介質 pH 值於栽培過程中逐漸下降的狀況於許多研究中皆有發現 (Ito & Lin 2008; Lai *et al*. 2015)。介質酸化的原因來自於肥料中銨態氮的吸收時，必須透過陽離子交換或 H^+ 的釋放所造成 (Maghrabi *et al*. 1985)。本試驗中小粒椰纖 pH 值下降幅度較大可能又與其含有泥炭土有關 (表 1)。以泥炭土為基質之培養土較易酸化，故常需添加苦土石灰或碳酸石灰調整 (Zhou *et al*. 2004)。本試驗中所使用的小粒椰塊中含有 50% 泥炭土，隨著種植時間的拉長逐漸酸化，因此加快介質酸化幅度。文心蘭於產業栽培上多以三分碎石作為介質進行栽培，碎石保水保肥能力差，業者常以高頻度施用彌補該問題，而本試驗之用肥方式參考文心蘭慣行操作手法，故推估保水性較強的椰塊於現行肥培手段下可能會吸收過多肥料，因此以椰塊進行文心蘭栽培時，須配合變更肥培操作方式，以延緩介質酸化速度。

物理特性上，尺寸大小對於介質保水能力與孔隙度皆造成明顯差異。孔隙度為通氣指標，除影響介質通氣之外，亦影響有效水分保有量與作物根系生長 (Chang 1987)。本試驗所使用之小粒椰塊因粒子較小且夾帶泥炭土，種植前孔隙度為 21.97%，介質較為緻密使得保水能力較高 (表 1)，隨著栽培時間推移，孔隙度下降，顯示椰塊發生分解或是結構崩塌，栽培 26 mo，介質呈現粉末狀幾乎無顆粒狀態 (圖 1C)，孔隙度因介質逐漸崩解為大小不一的顆粒互相填補而降低，保水能力反而因此增加。大粒椰纖於栽培 26 mo 後，孔隙度反而上升 (表 1)，推測大粒椰纖孔隙度大且崩解速度較慢，栽培過程中崩解之粉末隨澆水淋洗自空隙間流出，因而使孔隙度增加，但另一方面，椰纖結構因崩解而轉為鬆散，提供更多水分容納空間，因此也導致保水能力提升。

不同尺寸椰塊對文心蘭生育與切花品質之影響

本試驗以 2 種不同尺寸的椰塊進行文心蘭栽培測試，種植 2 mo 後可見新根長出 (圖 3)，但種植 20 mo 後小粒椰塊種植之文心蘭葉片生長開始出現弱勢，種植至 26 mo 時，假球莖亦明顯變小 (表 2)，同時可見根系因介質崩解而出現衰弱死亡的現象 (圖 1B)，推測小粒椰塊種植之文心蘭生長勢衰弱主要為介質崩解影響根系所導致。植物吸收養分以根部為主，本試驗中小粒椰塊崩解後，孔隙度下降導致介質內空氣不足，根系因而窒息死亡，影響養分吸收，葉面積縮小，進一步影響光合作用與光合成產物累積，造成假球莖變小 (表 2)。於產業上，文心蘭假球莖常被作為品質之良莠標準，假球莖具有貯存水分、碳水化合物以及營養元素的功能，為文心蘭生長與存活的重要貯藏器官 (He *et al*. 2011)。小粒椰塊介質之崩解所引發之連鎖反應，最終對於植株生長與切花產量及品質造成負面影響。

切花品質，以大粒椰塊處理組表現較佳，無論花梗、花序以及第一側枝長度均明顯較優 (表 3)。文心蘭切花通常以花梗長度、側枝數以及第一側枝長度作為分級標準，大粒椰塊種植文心蘭植株生產切花之 3–4 L 級 (主要外銷等級) 明顯較高，總產量表現上亦增加約 16.6% (表 4)。不同尺寸椰塊對於切花品質之影響，主要來自於種植後介質隨時間裂解，介質物化性的改變影響根系與地上部營養器官生長之結果。Hwang *et al*. (2022) 將草莓以不同比例的椰纖與椰粉進行混合，發現介質混合比例雖對營養生長無顯著差異，但會影響到草莓最終產量，故認為特定比例的椰纖混合介質，可有效增加介質孔隙度，增加蒸散作用、氣孔導度以及光合作用，進而促進產量的增加。不同尺寸椰塊對於花期並無顯著影響，兩處理於花期變化整體趨勢上接近，最高產量均出現在調查的第 3 個月 (圖 4)。

文心蘭切花目前為臺灣外銷最大宗切花，近年來面對主要輸出國日本對於品質要求逐漸升高，與其他國家如越南以及馬來西亞之競爭，穩質與穩量是文心蘭切花生產上的必備條件，以碎石為主之強疏水性栽培介質，配合無防雨能力簡易網室之傳統栽培模式，已不足以穩定滿足符合消費市場高品質要求，更無法於國際維持競爭力。椰塊之保水保肥能力，可使肥料

於介質中停留更長時間，增加植株吸收，提升環境資源使用效率，為有效之替代介質，其可生物降解的特性亦符合目前世界對於介質使用之期望。本試驗數據顯示，文心蘭植株選用較大尺寸規格的椰塊，並配合肥培模式之調整，可有效延長使用期限，於防雨設施內，替代碎石介質，維持植株生長與高品質切花生產。

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Effect of Coconut Chip Size on Growth and Cut Flower Yield of *Oncidium*

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Abstract

Lai, S. L., C. H. Chung, and T. E. Dai. 2024. Effects of coconut chip size on growth and cut flower yield of *Oncidium*. J. Taiwan Agric. Res. 73(3):197−206.

In this study, we utilized different sizes of coconut chips (50% 0.7−1.0 cm chip + 50% peat and 1.2−1.5 cm chip) in conjunction with an automatic drip irrigation system to cultivate the oncidium cut flower variety, 'Honey Angel', in a rainproof facility. The results showed that new roots grew normally within 2 mo of planting. However, after 20 mo of cultivation, the medium began to disintegrate, with smaller coconut chips disintegrating faster, almost completely turning into powder. Physical and chemical analyses of the medium revealed significant decreases in pH value and porosity. Regarding plant growth, there was no significant difference in the circumference of pseudo bulbs, except for the second leaf on contemporary pseudo bulbs, which was larger when cultivated with 1.2−1.5 cm coconut chips. Moreover, flowering quality was better when cultivated with larger-sized coconut chips, resulting in higher cut flower yield and quality. Overall, the results indicated that coconut chips could serve as a medium for producing oncidium cut flowers; however, larger-sized chips should be chosen to alleviate the negative effects of medium disintegration.

Key words: *Oncidium*, Alternative medium, Coconut chip, Flower quality.

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臺灣產真葉蟎屬 (蟎蜱亞綱：絨蟎目：葉蟎科) 種類的重新評估與證據釐清

何琦琛 [1,2] 廖治榮 [3,*]

摘要

何琦琛、廖治榮。2024。臺灣產真葉蟎屬 (蟎蜱亞綱：絨蟎目：葉蟎科) 種類的重新評估與證據釐清。台灣農業研究 73(3):207–217。

本研究針對臺灣地區真葉蟎屬 (蟎蜱亞綱：絨蟎目：葉蟎科) 物種的分類進行重新評估。過往臺灣的真葉蟎屬 (*Eutetranychus*) 長期以來均只記錄 1 種，即東方真葉蟎 (*E. orientalis* (Klein))，但近期在臺灣南部的木瓜園中發現了非洲真葉蟎 (*E. africanus* (Tucker)) 的存在，致使兩種真葉蟎的鑑定問題成為一待解決問題，亦即先前東方真葉蟎鑑定是否有問題。本研究利用真葉蟎調查與過往標本重新鑑定，特別是找尋文獻中的存證標本，旨在精確地鑑定臺灣產真葉蟎物種。研究總計共發現 230 隻雌性成蟎、144 隻雄性成蟎及 26 隻若蟎 (含第一若蟎與第二若蟎)。全部標本資訊則來自 43 筆採集樣本資訊，採集時間自 1966−2015 年。鑑定結果表明，包括先前文獻中存證標本在內的所有標本均與非洲真葉蟎的形態特徵相吻合，未發現任何符合東方真葉蟎特徵的標本。這一結果提出了一個新的假設：臺灣可能不存在東方真葉蟎。鑑於此一發現的重要性，本研究呼籲進行更廣泛的樣本蒐集與分析，並進一步整合分子資訊，以確定臺灣真葉蟎屬物種的實際分布情況。本研究的結果對於臺灣乃至全球的農業害蟎管理與生態研究具有深遠的意義，強調了進行物種精確鑑定的重要性與對於農業生態系統的長期監測的需求。

關鍵詞：形態鑑定、寄主植物多樣性、物種分布、分類學澄清、農業害蟎管理。

前言

真葉蟎屬 (*Eutetranychus*) 隸屬於葉蟎科 (Tetranychidae)、葉蟎亞科 (Tetranychinae) 的廣葉蟎族 (Eurytetranychini)，截至目前為止全世界記錄 36 種 (Migeon & Dorkeld n.d.)。真葉蟎屬體較扁平，體背毛匙狀，缺少爪間突爪 (empodial claw)，具 2 對側肛毛 (preanal setae) 與 2 對肛毛 (anal setae) (圖 1) (Meyer 1987; Tseng 1990; Ehara 1999; Kamran *et al*. 2018)。在部分國家，班克斯真葉蟎 (*E. banksi* (McGregor)) 與東方真葉蟎 (*E. orientalis* (Klein)) 分別危害南葡萄牙與西班牙的柑橘園，且這些物種在地中海盆地與南美洲的新地區定居，影響了當地的柑橘生產 (Ferragut *et al*. 2013; Naves *et al*. 2021)。在臺灣，真葉蟎屬物種過往雖不是主要農作物的關鍵害蟲 (Ho 2000)，但它們在觀賞植物上的大量發生已引起了人們的廣泛注意。特別是近年來，在屏東的網室木瓜園發現非洲真葉蟎 (*E. africanus* (Tucker)) 造成嚴重危害，進一步促使我們重新審視這一問題 (Ho *et al*. 2013, 2015; Lin *et al*. 2020)。

非洲真葉蟎的全球分布與寄主植物範圍顯示了其作為一種重要農業害蟲的潛力。根據最新 Spider Mites Web (Migeon & Dorkeld n.d.)，非洲真葉蟎已在非洲、亞洲以及太平洋

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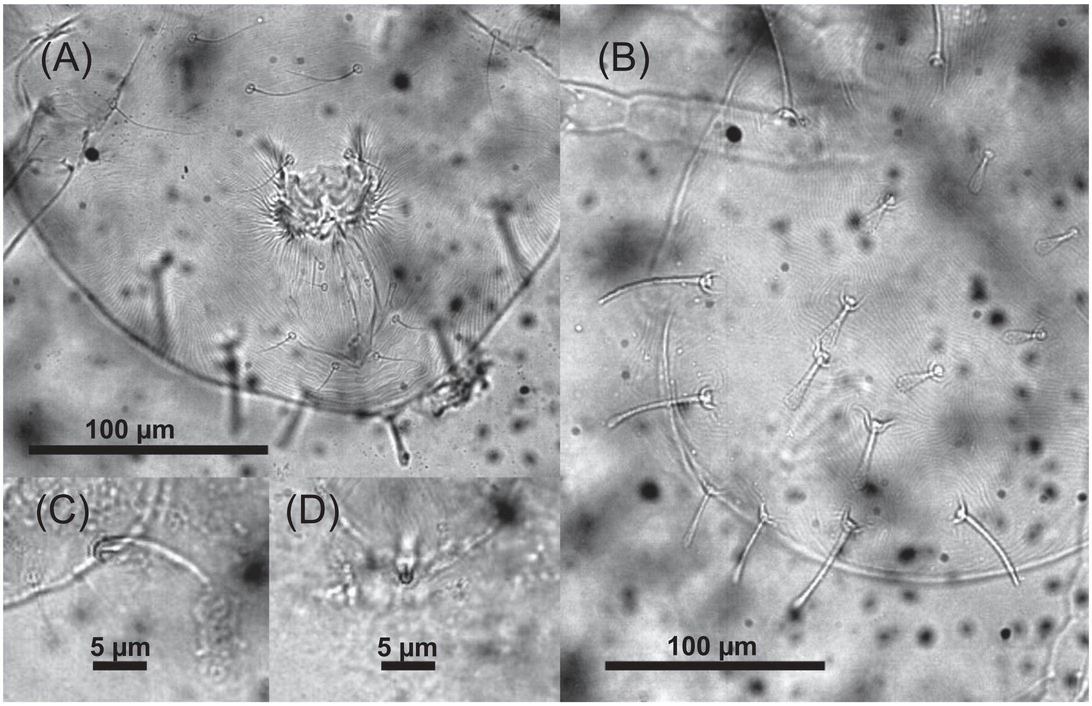

圖 1. 真葉蟎重要鑑定特徵。(A) 雌性腹面生殖區與肛門；(B) 雌性背面背毛匙狀；(C) 非洲真葉蟎雄性陽莖側面觀；(D) 非洲真葉蟎雄性陽莖玻片製作失敗。

Fig. 1. Key identification characters of genus *Eutetranychus*. (A) Ventral view of the female genital region and anus; (B) dorsal view of female showing the spoon-shaped dorsal setae; (C) lateral view of the male aedeagus of *E. africanus*; and (D) failed slide preparation of the male aedeagus of *E. africanus*.

島嶼國家，如巴布亞紐幾內亞有所記錄。這種真葉蟎已知的寄主植物範圍約 23 科 100 種，從經濟重要的作物如木瓜 (*Carica papaya* L.)、柑橘 (*Citrus* spp.) 及葡萄 (*Vitis* spp.)，到觀賞植物如夾竹桃 (*Nerium* spp.) 與緬梔 (*Plumeria* spp.) 等。這一害蟲在其大量發生並成為主要害蟲的地區，對農業生產構成顯著威脅。非洲真葉蟎的廣泛分布與多樣化的寄主植物範圍凸顯其對農業作物與園藝作物的潛在風險，也有可能成為重要的入侵生物。

在過去幾十年中，臺灣對於真葉蟎屬的物種鑑定一直存在混淆 (e.g., Lo 1965, 1968, 1969; Lo & Hsia 1968; Tseng 1990; Ho 2000)。Lo (1965) 首次記錄班克斯真葉蟎 (柑橘褐蜘蛛)，而後其報導東方真葉蟎的存在，並指出先前班克斯真葉蟎為錯誤鑑定 (Lo & Hsia 1968; Lo 1969)。由於過往真葉蟎屬在臺灣僅記錄有 1 種，採得之標本未仔細觀察種級鑑定特徵，屬級特徵相符後均視為東方真葉蟎。這一挑戰不僅局限於臺灣，而是一個全球性的問題。尤其是東方真葉蟎與其近緣種間的鑑定，由於形態上的顯著種內變異，一直是蟎蜱分類學中最具挑戰性的問題之一，與二點葉蟎 (*Tetranychus urticae* Koch) 種群的辯論相媲美 (Auger *et al*. 2013)。然而與二點葉蟎的研究相比，東方真葉蟎的研究未受到等同的重視，可能是因為它在農業上沒有如後者般視為主要害蟲。但這一物種展示出的種內變異卻超過葉蟎屬的其他物種，這使得它的種內變異成為一個複雜的問題，已知它至少有 4 個同物異名，而且還可能有更多，這有待進一步的研究確認 (Meyer 1987; Kamran *et al*. 2018)。大約在 1990 年代後期，後藤哲雄教授 (Gotoh personal communication) 對筆者 (第一作者) 提到，臺灣與日本的東方真葉蟎長久以來均為錯誤鑑定 (e.g., Tseng 1990; Ehara 1999)，實際上應

為非洲真葉蟎。此兩種形態學上的微小差異，如第二足基節腹面的毛數，雖是區分這兩種真葉蟎的關鍵特徵 (Meyer 1987)，可能受限於鑑定者經驗與顯微鏡品質，在實際鑑定中往往被忽視。

本研究旨在結合作者調查真葉蟎與檢視標本館過往蒐集的臺灣產真葉蟎屬標本，解決國內長期存在的物種鑑定問題。鑑於目前無法獲得分子證據來識別過往的存證標本，我們主要依據形態學的廣泛概念進行物種鑑定 (Meyer 1974, 1987; Toroitich *et al*. 2009; Kamran *et al*. 2018)。本研究將比對館藏樣本，以形態特徵重新鑑定，確認所有標本種名、寄主植物及採集地等關係能確實結合。藉此研究，期能釐清臺灣田間真葉蟎屬物種身分，瞭解真葉蟎的生物多樣性與分布重要資訊，同時也有助於為後續研擬害蟎管理資訊，提供基礎資料。

材料與方法

本研究包含作者野外調查真葉蟎與檢視標本館過往蒐集的臺灣產真葉蟎屬標本。真葉蟎野外調查係以放大鏡檢查寄主植物之葉面與葉背，確認有發現後帶回實驗室進一步以解剖顯微鏡鏡檢並製作玻片。寄主植物以 Spider Mite Webs 記錄之資訊進行調查參考 (Migeon & Dorkeld n.d.)。此外亦有蒐集土壤樣本，並以伯氏漏斗 (Berlese funnel) 分離 7 d，取得樣本後存於酒精內以進行後續玻片標本製作。玻片標本依照個體狀況判斷是否以乳酸透化，後續以何氏液 (Hoyer's medium) 封片 (Liao *et al*. 2020)，雄性個體製作側面玻片以觀察陽莖，加熱乾燥 7 d 後保存。玻片標本保存位於臺中市霧峰區臺灣蟎蜱研究室 (Taiwan Acari Research Laboratory; TARL)。關於過往標本檢查部分，係針對農業部農業試驗所 (以下簡稱農試所) (Taiwan Agricultural Research Institute; TARI) 應用動物組館藏的真葉蟎屬標本，進行重新檢視。此外並期望能重新檢視過往文獻如 Lo (1965, 1968, 1969) 與 Lo & Hsia (1968) 之東方真葉蟎的存證標本。玻片標本使用微分干涉相差顯微鏡對標本進行鑑定，並根據可見的形態特徵對標本進行分類。根據 Meyer (1974, 1987)、Toroitich *et al*. (2009) 以及 Kamran *et al*. (2018) 的形態描述。特別關注的關鍵鑑定特徵包括第二基節上的剛毛數量與雄性陽莖形狀等。本研究並提供重要鑑定特徵的手繪圖與玻片照以供參考。圖像進一步使用Adobe Photoshop 2021 (Adobe Systems Inc., San Jose, CA, USA) 進行細化，以確保準確呈現。此外並使用 QGIS (QGIS.ORG 2023) 繪製所有樣本採集分布地圖，以展示本研究的物種地理分布情況。此外熱圖分析是在 RStudio (版本 2023.09.1-494) (RStudio Team 2023) 中使用 R (版本 4.3.2) (R Core Team 2023) 進行的，採用 "ggplot2" (Wickham *et al*. 2019) 與 "reshape2" (Wickham 2020) 套件來進行真葉蟎數量與寄主植物科級與屬級之關係分析。所有詳細的鑑定數據與分析結果均在附錄，包括每個標本的詳細鑑定紀錄、寄主植物資訊以及相關的地理位置數據。

結果

在本研究中，我們調查與重新檢視臺灣真葉蟎屬物種玻片標本。總計發現 230 隻雌性成蟎、144 隻雄性成蟎及 26 隻若蟎 (含第一若蟎與第二若蟎)。其中採集部分有 61 隻雌性成蟎、60 隻雄性成蟎及 1 隻若蟎；而農試所館藏 169 隻雌性成蟎、84 隻雄性成蟎及 25 隻若蟎。全部標本資訊則來自共 43 筆採集樣本資訊，其中調查部分共有 27 筆，農試所有 16 筆。採集地主要集中於臺灣的中南部，僅有早期標本採於臺北，桃園、新竹、苗栗、宜蘭及花蓮無發現，此外這些樣本中並無於離島發現真葉蟎 (圖 2)。標本採集時間最早自 1966 年起，最晚到 2015 年。關於寄主植物部分，除土壤與無寄主鑑定資訊的樣本外，其餘樣本總計有 17 科、26 屬及 25 種，並包括部分樣本寄主植物僅鑑定至屬級 (附錄)。而最多真葉蟎的寄主植物科為芸香科 (Rutaceae) 共 191 件個體，其次為桑科 (Moraceae) 共 29 件個體及樟科 (Lauraceae) 共 23 件個體。寄主植物屬級部分，個體數最多的為柑橘屬 (*Citrus* sp.) 168 件個體，其次為鱷梨屬 (*Persea* sp.) 23 件個體。種級最多則為酪梨 (*Per. americana* Mill.)

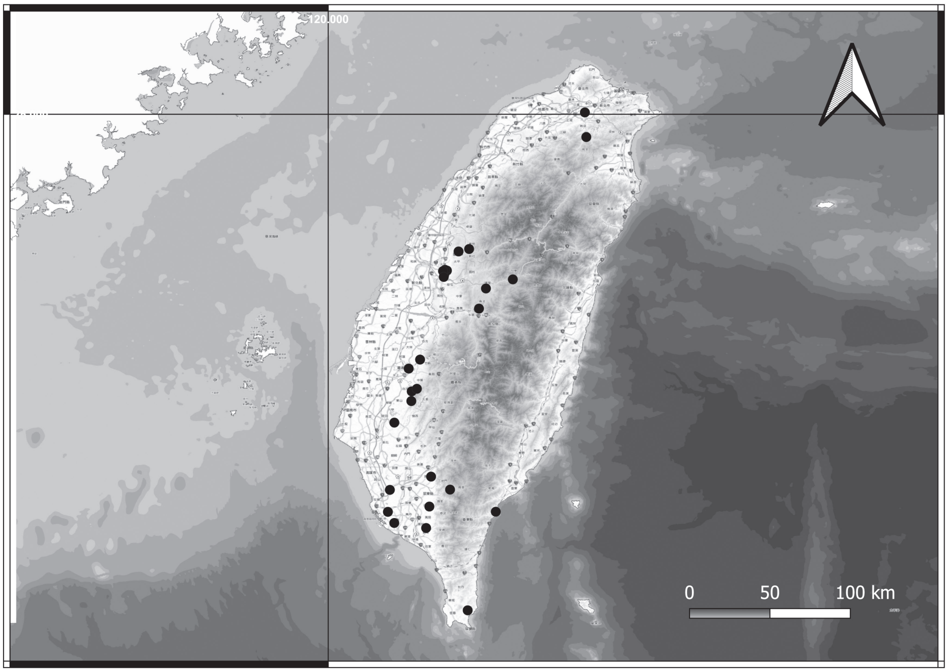

圖 2.　本研究中真葉蟎的採集地點地圖。圓圈表示採集地點。

Fig. 2.　Collection site maps for *Eutetranychus* mites in the present study. Circles indicate the collection sites.

23 件、木瓜 (*C. papaya* L.) 18 件及榴槤 (*Durio zibethinus* L.) 18 件個體 (圖 3)。農試所應用動物組標本館中找到採集時間為 1967 年的標本，其採集資料與 Lo & Hsia (1968) 報導的 17 筆資料中的 12 筆相吻合，也包括 Lo (1968) 報導的梨樹 (*Pyrus pyrifolia* (Burm.f.) Nak.)，為該報告之存證標本。

針對東方真葉蟎與非洲真葉蟎的形態鑑定，係於光學顯微鏡下檢視雄性陽莖彎曲角度與第二足基節腹面上的毛是 2 根或 1 根 (圖 4)。經過詳細的樣本檢視發現，所有樣本中，包含採集與館藏標本，其先前歸類為東方真葉蟎的所有標本，均屬於非洲真葉蟎。這一發現基於對來自臺灣各個地區與各種寄主植物上蒐集的數十個標本的詳細檢查。無論標本是雌性、雄性還是若蟎階段，它們的特徵均與非洲真葉蟎相符，而與東方真葉蟎不符。這些結果表明：過去對於臺灣產真葉蟎屬物種的鑑定有誤。非洲真葉蟎實際於臺灣廣泛分布，而東方真葉蟎的存在存疑。本研究的發現為改正臺灣真葉蟎屬物種紀錄提供了重要依據，並對未來的農業害蟎管理策略與生態研究具有重要意義。

討論

在臺灣，非洲真葉蟎首次受到關注是由 Ho *et al*. (2013) 記錄，當時它已成為南部木瓜園的重要害蟲，爾後又有 Ho *et al*. (2015) 與 Lin *et al*. (2020) 報導非洲真葉蟎，因此促使我們重新審視其鑑定與分布狀況。本研究發現，目前紀錄多於中南部為主，在北部、東部及離島並未發現非洲真葉蟎 (圖 2)，這部分可能為調查頻率與強度的不足，後續尚需努力與植物保護同仁協助。然而，這一觀察也可能暗示，相較於南部的炎熱天氣，這些地區的氣候條件可能不利於非洲真葉蟎的大量繁衍成為重要害蟎。然而這個推論也值得注意，檢查過往標本時發現 (Lo 1965, 1968, 1969; Lo & Hsia 1968)，其曾在臺北的果樹上記錄到這一物種。因此未發現的地區究竟是不適合其生存，或是

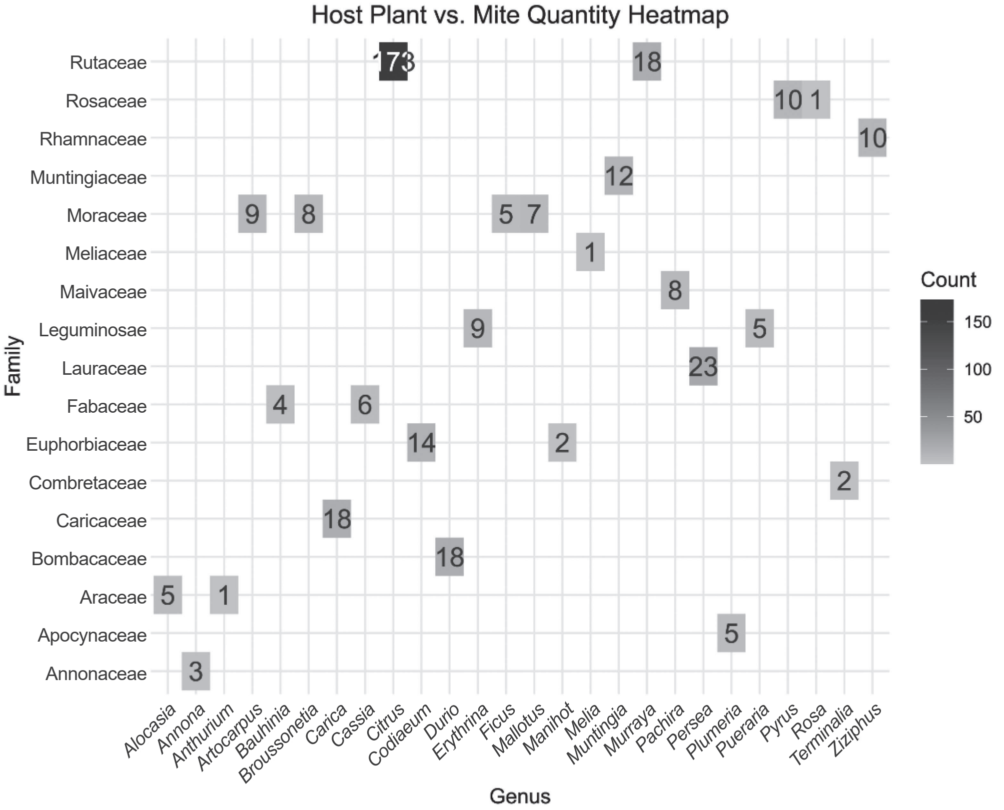

圖 3. 寄主植物與真葉蟎數量之間的熱力圖。Y 軸代表寄主植物科，X 軸代表寄主植物屬，方框內的數字表示相對應的真葉蟎數量。

Fig. 3. Heatmap representing the relationship between host plants and mite quantities. The Y-axis denotes the plant families, and the X-axis denotes the plant genera. The numbers within the squares indicate the corresponding mite quantities.

反映了氣候變化導致的害蟎相更替，亦或是由於北部都市化導致耕地減少，進而使得適合的寄主植物變得稀少，導致棲地破碎化 (Liao *et al*. 2020)，是一個值得深入探討的問題。這些發現強調了進行更全面的地區性調查與長期監測的重要性，以確定非洲真葉蟎在臺灣的確切分布情況及其隨時間的變化。

在臺灣，我們的研究發現非洲真葉蟎主要發生在芸香科、桑科及樟科等植物上 (圖 3)。根據 Spider Mites Web 的最新資料 (Migeon & Dorkeld n.d.)，這物種的寄主植物範圍遠遠超出這些科別。就經濟作物而言，我們在臺灣主要發現非洲真葉蟎於木瓜與柑橘屬，而這些寄主在 Spider Mite Web 的列表中也有記載。然而應小心考慮資料庫中的信息，因為來自不同的文獻，可能包含一定程度的鑑定錯誤，這可能會導致相關信息的不準確性。特別是對於臺灣未觀察到的寄主植物，如茄科 (Solanaceae) 的茄子 (*Solanum melongena* L.)，也可能是臺灣產的茄子不適合其生存，潛在風險仍須注意。對於園藝作物，例如馬拉巴栗 (*Pachira aquatica* Aublet)，儘管它們在經濟上的重要性可能不如柑橘或木瓜，但作為非洲真葉蟎的寄主植物，它們展現了這類害蟲對多樣化生態環境的適應性。此外，其也可能通過園藝盆栽的方式被人為帶入其他地區，成為一種潛在的儲存寄主植物 (reservoir host plant)。總體來說，非洲真葉蟎在國內的寄主植物範圍與資料庫中

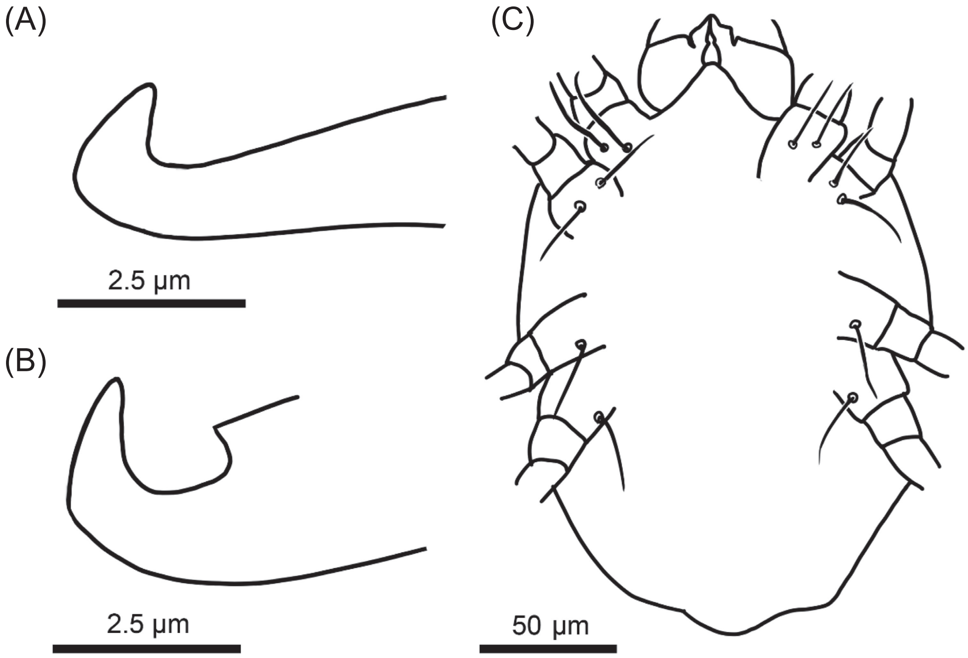

圖 4. 非洲真葉蟎與東方真葉蟎重要鑑定特徵比較。(A) 非洲真葉蟎雄性陽莖；(B) 東方真葉蟎雄性陽莖 (修改自 Meyer (1974) 與 Toroitich *et al*. (2009))；(C) 非洲真葉蟎腹面足基節毛數。

Fig. 4. Comparison of diagnostic characters between *Eutetranychus africanus* and *E. orientalis*. (A) Male aedeagus of *E. africanus*; (B) male aedeagus of *E. orientalis* (modified from Meyer (1974) and Toroitich *et al*. (2009)); and (C) number of coxal setae on the ventral view of *E. africanus*.

相關性高，這證明了它們作為強適應性害蟲的能力，能在各種植物上生存與繁衍也反映其成為重要害蟎的潛力。

在臺灣，關於真葉蟎的研究歷程顯示了鑑定工作的進步與挑戰。早期由 Lo (1965, 1968, 1969) 與 Lo & Hsia (1968) 報導的東方真葉蟎，涵蓋了廣泛的寄主植物範圍，包括月橘 (*Murraya paniculata* (L.) Jack)、蓖麻 (*Ricinus communis* L.) 及馬拉巴栗等。然而本研究的結果指出，這些早期紀錄中的東方真葉蟎，實際上屬於非洲真葉蟎。這一發現不僅糾正了過去對於真葉蟎種群身分的誤解，也凸顯了物種鑑定中形態學特徵的重要性，特別是早期研究中往往忽略了基節上的毛數這一關鍵形態學特徵 (圖 4) (Auger *et al*. 2013)。相較之下，本研究通過細緻的形態學分析，確認在臺灣的真葉蟎主要為非洲真葉蟎，而非早期文獻中所提及的東方真葉蟎。這一結論基於對標本的重新檢視，包括 Lo (1968) 蒐集並報導位於臺北的梨樹上的標本，這為我們提供了寶貴的歷史數據。此外，Tseng (1990) 雖提供了重要的鑑定特徵描述與形態繪圖，但其研究中未特別強調基節毛數的觀察。遺憾的是，由於其標本已不可再檢視 (Anonymous 2009; Liao *et al*. 2017, 2020)，對其記錄的東方真葉蟎的種類身分確認存在困難。然而本研究在 Tseng (1990) 報導的寄主植物上 (榕屬 *Ficus* spp.) 確認了非洲真葉蟎的存在，初步支持過去紀錄為錯誤鑑定。綜上所述，本研究不僅糾正了關於真葉蟎在臺灣分布與種群身分的歷史誤解，也凸顯了持續更新與檢視歷史數據的必要性。

基於本研究的結果，我們提出假設：『臺灣可能僅存有非洲真葉蟎，而無東方真葉蟎。』未來研究應加強對臺灣未充分調查地區的調查

工作，特別是在氣候及生態條件與南部大相逕庭的北部、東部及離島地區。這些地方的數據在現有研究中相對欠缺，進一步的調查可能揭示新的種群與寄主植物關係，豐富我們對於真葉蟎物種多樣性與分布範圍的瞭解。此外，考慮到氣候變遷與都市化對生態環境的影響，未來的研究應評估這些因素如何塑造害蟲分布與寄主植物選擇。這將對準確評估非洲真葉蟎對臺灣農業生態系統的影響與制定相應的害蟲管理策略具有重要價值。隨著物種鑑定工作的進步，對形態學特徵的細緻研究顯得尤為重要。因此，我們應持續提升鑑定技術，並建議未來的研究應整合分子特徵資料，利用如 DNA 條碼技術，以確定真葉蟎屬物種的精確分類，從而提高鑑定的準確性與可靠性。目前國內已有的真葉蟎分子證據是 Matsuda *et al*. (2014) 於臺中採自葛藤 (*Pueraria montana* (Lour.) Merr.) 的非洲真葉蟎。最後本研究呼籲學術界、農業部門與環境保護組織合作，共同促進對真葉蟎及其對農業生態系統影響的深入研究。透過跨學科與跨部門的合作，我們能夠更有效地應對害蟲挑戰，保護農業生產的可持續性與豐富的生物多樣性。

誌謝

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附錄． 真葉蟎標本採集資訊。

Appendix. Collection information of *Eutetranychus* mites[z].

Specimen depository	Date	City	District	Location	Host plant	Collector	Adult female	Adult male	Protonymph
TARI	1980/11/12	Taichung	Wufeng	Wanfeng	*Ficus* sp.	P. K. C. Lo	2	2	0
TARI	1991/10/14	Taichung	Wufeng	TARI	*Pyrus pyrifolia*	P. K. C. Lo	1	2	0
TARI	1966/12/18	Taipei			*Murraya paniculata*	P. K. C. Lo	3	0	2
TARI	1967/1/26	Taipei			*Mur. paniculata*	L. S. Lu	7	2	0
TARI	1967/4/13	Taipei			*Persea americana*	E. S. Lu	13	5	5
TARI	1967/4/16	Taipei			*Artocarpus integer*	E. S. Lu	6	2	1
TARI	1967/4/27	Taipei			*Mur. paniculata*	E. S. Lu	2	2	0
TARI	1967/4/27	Taipei			*Annona squamosa*	E. S. Lu	1	1	1
TARI	1967/9/19	Taipei			*Py. pyrifolia*	P. K. C. Lo	1	1	0
TARI	1967/9/19	Taipei			*Citrus* sp.	P. K. C. Lo	28	10	0
TARI	1967/2/27	Pingtung			*Durio zibethinus*	P. K. C. Lo	13	4	1
TARI	1967/2/27	Pingtung			*Muntingia calabura*	P. K. C. Lo	10	2	0
TARI	1966/12/14	Kaohsiung	Fengshan		*Pachira aquatica*	P. K. C. Lo	7	0	1
TARI	1997/1/6	Kaohsiung	Dashe		*Ziziphus mauritiana*	P. K. C. Lo	3	7	0
TARI	1967/4/30	New Taipei	Wulai		*Cit.* sp.	E. S. Lu	50	28	14
TARI	1967/9/8	New Taipei	Wulai		*Cit.* sp.	P. K. C. Lo	22	16	0
TARL	2001/3/29	Taichung	Wufeng	TARI	*Anthurium andraeanum*	C. C. Ho	1	0	0
TARL	2003/11/30	Taichung	Wufeng	Mt. Azhaowu	*Carica papaya*	C. C. Ho	1	2	0
TARL	2003/2/23	Taichung	Taiping	Zhongzheng Camp Zone	soil	C. C. Ho	1	6	0
TARL	2003/7/7	Taichung	Wufeng	Mt. Azhaowu	soil	C. C. Ho	1	0	0
TARL	2008/1/20	Taichung	Wufeng	921 earthquake museum of Taiwan	*Erythrina* sp.	C. C. Ho	3	3	1
TARL	2009/9/23	Taichung	Dongshi	Qingfu Village	*Py. pyrifolia*	C. C. Ho	1	4	0
TARL	2012/10/20	Taichung	Wufeng		unknown plant	C. C. Ho	2	1	0
TARL	2012/3/10	Taichung	Wufeng	on street	*Codiaeum variegatum*	C. C. Ho	6	5	0
TARL	2014/12/28	Taichung	Wufeng		*Broussonetia papyrifera*	C. C. Ho	4	4	0
TARL	2014/4/18	Taitung	Taimali		*Cassia fistula*	Y. T. Hsu	3	3	0
TARL	2005/12/19	Tainan	Baihe	Guanziling	*Alocasia odora*	C. C. Ho	3	2	0

附錄． 真葉蟎標本採集資訊。(續)

Appendix. Collection information of *Eutetranychus* mites[z]. (continued)

Specimen depository	Date	City	District	Location	Host plant	Collector	Adult female	Adult male	Protonymph
TARL	2005/12/19	Tainan	Baihe	Guanziling	*Cod. variegatum*	C. C. Ho	2	1	0
TARL	2005/12/19	Tainan	Baihe	Guanziling	*Mallotus paniculatus*	C. C. Ho	4	3	0
TARL	2007/1/30	Tainan	Dongshan	Qingshan Village	unknown plant	C. C. Ho	1	1	0
TARL	2010/1/9	Tainan	Baihe	Guanziling	*Manihot esculenta*	C. C. Ho	1	1	0
TARL	2011/3/19	Tainan	Danei		*Pueraria montana*	C. C. Ho	0	1	0
TARL	2000/11/23	Nantou	Renai	Renzhiguan	unknown plant	C. C. Ho	5	5	0
TARL	2010/3/22	Nantou	Puli		*Rosa multiflora*	C. C. Ho	0	1	0
TARL	2015/11/17	Nantou	Yuchi	Sun Moon Lake	*Plumeria obtusa*	C. C. Ho	3	2	0
TARL	2001/7/7	Pingtung	Manzhou		*Pu. montana*	C. C. Ho	3	1	0
TARL	2001/8/10	Pingtung	Neipu	NPUST	*Ficus benjamina*	C. C. Ho	0	1	0
TARL	2003/5/9	Pingtung	Wutai		*E. variegata*	W. H. Chen	0	2	0
TARL	2014/4/17	Pingtung	Gaoshu		*Car. papaya*	C. C. Ho	9	6	0
TARL	2012/10/27	Kaohsiung		Fongshan Reservoir	*Bauhinia variegata*	C. C. Ho	2	2	0
TARL	2012/10/28	Chiayi City		Lantan	*Terminalia catappa*	C. C. Ho	0	2	0
TARL	2001/2/8	Chiayi County	Zhuqi	Zhuqi Park	*Cit. maxima*	C. C. Ho	5	0	0
TARL	2010/7/12	Chiayi County	Zhongpu	Dongzijiao	*Melia azedarach*	C. C. Ho	0	1	0

[z] TARI: Taiwan Agricultural Research Institute; TARL: Taiwan Acari Research Laboratory; NPUST: National Pingtung University of Science and Technology.

Reassessment and Clarification of *Eutetranychus* Species (Acari: Trombidiformes: Tetranychidae) in Taiwan

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Abstract

Ho, C. C. and J. R. Liao. 2024. Reassessment and clarification of *Eutetranychus* species (Acari: Trombidiformes: Tetranychidae) in Taiwan. J. Taiwan Agric. Res. 73(3):207−217.

This study conducts a comprehensive reassessment of the classification of the *Eutetranychus* genus (Acari: Trombidiformes: Tetranychidae) in Taiwan. Historically, only 1 species of this genus, the oriental spider mite (*E. orientalis*), has been recorded in Taiwan. However, recent discoveries of the African spider mite (*E. africanus*) in papaya orchards in southern Taiwan have raised questions about the identification of these two spider mite species, specifically whether previous identifications of the oriental spider mite may have been incorrect. By utilizing surveys of spider mites and re-examining past specimens, especially those documented in the literature as voucher specimens, this study aims to accurately determine the species identification of *Eutetranychus* in Taiwan. The research identified a total of 230 female adult mites, 144 male adult mites, and 26 nymphal mites (including both protonymphs and deutonymphs) from 43 collection records spanning from 1966 to 2015. The results indicate that all specimens, including those previously documented in the literature as oriental spider mites, match the morphological characteristics of the African spider mite, with no specimens found to exhibit characteristics of the oriental spider mite. This finding proposes a new hypothesis: The oriental spider mite may not exist in Taiwan. Given the significance of this discovery, the study calls for more extensive sample collection and the integration of morphological identification with molecular data to ascertain the actual distribution of *Eutetranychus* species in Taiwan. The findings of this research have profound implications for agricultural pest management of mites and ecological studies in Taiwan and globally, underscoring the importance of accurate species identification and the need for long-term monitoring of agricultural ecosystems.

Key words: Morphological identification, Host plant diversity, Species distribution, Taxonomic clarification, Agricultural pest management of mites.

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Short Communication

Total Phenolic Contents and Antioxidant Activity in Sweet Potato Leaves and Carrots after Steam-Cooking

Chien-Ya Hung[1,*], Ya-Ru Jiang[2], and Yue-Chen Chen[2]

Abstract

Hung, C. Y., Y. R. Jiang, and Y. C. Chen. 2024. Total phenolic contents and antioxidant activity in sweet potato leaves and carrots after steam-cooking. J. Taiwan Agric. Res. 73(3):219–223.

Vegetables serve as an important source of natural antioxidants owing to the presence of phenolic compounds, which provide health benefits. In this study, we investigated the effect of steam-cooking on the total phenolic contents (TPC) and antioxidant activity, as assessed by oxygen radical absorbance capacity (ORAC), in sweet potato leaves (SPL) and carrots (CA). The results revealed an increase in TPC and antioxidant activity in the steamed stems and leaves of SPL. However, while TPC and antioxidant activity increased in the steamed peel of CA, there was a slight decrease in the steamed flesh of CA. A positive correlation was observed between TPC and antioxidant activity. According to the study, it is suggested that employing a suitable steam-cooking process could serve as a preferable method to enhance or preserve the TPC and antioxidant activity of SPL and CA. Further research is needed to elucidate the role of individual bioactive components influencing the antioxidant properties of SPL and CA.

Key words: Sweet potato leaves, Carrot, Total phenolic contents, Antioxidant activity.

The consumption of vegetables plays an important role in maintaining our body's health. Vegetables act as a good source of natural antioxidants due to the occurrence of phenolic compounds, such as flavonoids and phenolic acids (Donglin & Hamauzu 2004). The antioxidant properties of these compounds contribute to the reduction of oxidative stress, thus aiding in the prevention of chronic diseases (Muscolo *et al*. 2024). Most vegetables are cooked before consumption because cooking is an important step in obtaining safe and hygienic vegetables. Through the process of heating, vegetables undergo chemical reactions that enhance their digestibility and nutritional values. Several studies have reported that heating processes cause many changes in the chemical composition of vegetables. After cooking, the total polyphenol content (TPC) and antioxidant activity in various vegetables could either increase or decrease compared to fresh vegetables (Faller & Fialho 2009). Previous study indicated that steaming is a proper thermal processing method to preserve these phytochemicals and affect the antioxidant activity in vegetables (Palermo *et al*. 2014; Nayak *et al*. 2015).

Taiwan, being a tropical country, boasts a diverse array of vegetables. However, due to dietary preferences, most of these vegetables

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undergo cooking processes rather than being consumed raw. Despite this, there is limited information available regarding the impact of steaming on the TPC and antioxidant activity of vegetables cultivated in Taiwan. Sweet potato leaves (SPL) and carrots (CA) are among the commonly consumed leafy and root vegetables in Taiwan. It is important to understand the extent of TPC or antioxidant capacity loss in steamed vegetables compared to their levels in fresh vegetables. Therefore, the objective of this study was to evaluate the influence of steam-cooking on the TPC and antioxidant activity of various edible parts in SPL and CA.

The SPL and CA specimens were procured from a local supermarket in Kaohsiung, Taiwan. Subsequently, the leaf and stem tissues of SPL were meticulously separated using a knife, while the peel and flesh tissues of CA were delineated using a peeler and knife, respectively. All sections of total 2 kg of SPL and 2 kg of CA were diced into small pieces. One kilogram of the chopped SPL and CA was subjected to steaming over boiled water for 3 min, while the remaining portions were left uncooked. Following this, both the steamed and raw samples were dried in an oven at 55℃ for 16 h and subsequently ground using a commercial grinder. Five grams of the ground powders from the peel and flesh of CA, as well as from the leaf and stem of SPL, were accurately weighed and immersed in 45 mL of distilled water for 30 min with periodic agitation. Subsequently, the samples were sonicated for 30 min at room temperature. The resultant mixtures were then subjected to centrifugation at 2,500× *g* for 10 min, following which the supernatants were collected and stored at -20℃ for subsequent analysis.

The TPC of the SPL and CA extracts was determined by the Folin-Ciocalteu method (Singleton *et al*. 1999) with slight modifications. TPC values were expressed as milligram of gallic acid equivalent (GAE) per 100 g of fresh sample and are presented in Table 1. In raw SPL, the TPC in leaves was determined to be 70.48 mg GAE per 100 g fresh weight (fw), which was 2.1 times higher than that in stems. Following steaming, the TPC of SPL significantly increased to 312.21 mg GAE per 100 g fw ($P < 0.05$) compared to raw leaves. The TPC in leaf fractions of steamed SPL exhibited a 4.5-fold increase compared to raw leaves, and a significant increase was also observed in stem fractions of steamed SPL. These findings align with previous research indicating a significant increase in TPC in steamed SPL (Sun *et al*. 2014). In raw CA, the TPC in peel was measured at 27.88 mg GAE per 100 g fw, which was 1.4 times higher than that in flesh. Following steaming, the TPC in the peel increased to 45.15 mg GAE per 100 g fw, while no significant difference was observed in the TPC of CA flesh. Different tissues in different vegetables contain various compounds, some

Table 1. The total phenolic content in different raw and steamed sweet potato leaves (SPL) and carrots (CA) tissues.

Tissues of SPL and CA	Total phenolic content[z] (mg GAE 100 g^{-1} fresh weight)
Stem fractions of raw SPL	33.30 ± 0.39 e[y]
Stem fractions of steamed SPL	37.27 ± 1.68 d
Leaf fractions of raw SPL	70.48 ± 5.37 b
Leaf fractions of steamed SPL	312.21 ± 1.62 a
Peel fractions of raw CA	27.88 ± 0.53 f
Peel fractions of steamed CA	45.15 ± 1.52 c
Flesh fractions of raw CA	19.83 ± 0.48 g
Flesh fractions of steamed CA	16.52 ± 1.09 g

[z] GAE: gallic acid equivalent.
[y] Mean ± standard deviation ($n = 3$). Means within the column followed by the different letters are significantly different at $P < 0.05$ by Duncan's multiple range test.

of which are thermally resistant, and some are not; therefore, the same cooking method may have different effects on different kinds of vegetables (Bernhardt & Schlich 2006).

The antioxidant activity of SPL and CA was evaluated using the oxygen radical absorbance capacity (ORAC) assay, as described in a previous study (Ninfali *et al*. 2002). The ORAC values representing the antioxidant activity of SPL and CA are depicted in Fig. 1. The highest ORAC value was observed in leaf fractions of steamed SPL (955.72 µmol trolox equivalent (TE) 100 g^{-1} fw), while the lowest ORAC value was showed in the flesh of steamed CA (45.12 µmol TE 100 g^{-1} fw). The ORAC value in the leaf fractions of raw SPL was higher than that in the stem fractions. Steaming led to an increase in the antioxidant activity of SPL. Specifically, the ORAC value in steamed SPL leaves was 2.5 times higher than that in raw SPL leaves, which correlated with the increase in TPC. Moreover, the ORAC value was higher in the peel and lower in the flesh of both raw and steamed CA. The ORAC value in the peel increased to 145.63 µmol TE per 100 g^{-1} fw, whereas a decrease in the ORAC value was observed in the flesh of CA after steaming. The ORAC values exhibited a similar pattern to the TPC in both SPL and CA tissues. The increase in TPC corresponded with the trend in antioxidant activity, suggesting that TPC might be the primary contributor to the antioxidant activity. Furthermore, the results indicated that TPC had a positive correlation with the ORAC value in both raw (r = 0.86) and steamed (r = 0.99) SPL and CA (Tables 2–3).

This study showed that steaming could enhance or maintain TPC and antioxidant activity of SPL and CA. It is suggested that utilizing an appropriate steam-cooking method may be a preferred approach to preserve the TPC and antioxidant activity of SPL and CA. Steam-cooking might impact the antioxidant activity of vegetables by facilitating the release of phenolic compounds, as heating can disrupt these structures, thereby releasing bound phenolic compounds and increasing phenolic contents (Nayak *et al*. 2015). It is possible that heating help break the cell wall and release antioxidant compounds, leading to an increase in antioxidant capacity (Choi *et al*. 2006). Nonetheless, further research is required to elucidate the role of individual bioactive components that influence the antioxidant properties of SPL and CA.

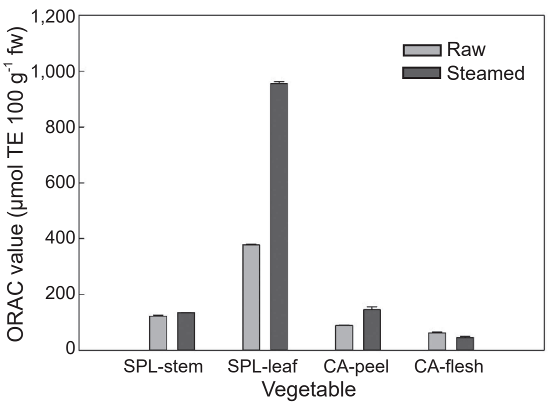

Fig. 1. The oxygen radical absorbance capacity (ORAC) value in different raw and steamed sweet potato leaves (SPL) and carrots (CA) tissues. The error bars indicate the standard deviation of triplicate analysis. TE: trolox equivalent.

Table 2. Pearson correlation coefficients (r) between total phenolic content (TPC) and oxygen radical absorbance capacity (ORAC) value of raw sweet potato leaves (SPL) and carrots (CA).

Variables	TPC	ORAC
TPC	1	
ORAC	0.86*	1

*The correlation coefficients are significant at $P < 0.05$.

Table 3. Pearson correlation coefficients (r) between total phenolic content (TPC) and oxygen radical absorbance capacity (ORAC) value of steamed sweet potato leaves (SPL) and carrots (CA).

Variables	TPC	ORAC
TPC	1	
ORAC	0.99*	1

*The correlation coefficients are significant at $P < 0.05$.

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蒸煮甘薯葉和紅蘿蔔總酚含量與抗氧化活性

洪千雅 [1,*] 蔣雅如 [2] 陳玥辰 [2]

摘要

洪千雅、蔣雅如、陳玥辰。2024。蒸煮甘薯葉和紅蘿蔔總酚含量與抗氧化活性。台灣農業研究 73(3):219−223。

蔬菜含有對身體健康有益的酚類化合物，也是天然抗氧化劑的重要來源。本研究初步探討蒸煮加熱對甘藷葉與紅蘿蔔的總酚含量及抗氧化活性的影響。試驗結果顯示，甘藷葉經蒸煮後，其莖、葉的總酚含量與抗氧化活性均有所增加；而紅蘿蔔在蒸煮後，紅蘿蔔皮的總酚含量與抗氧化活性皆增加，但在果肉部分則略微降低。甘藷葉與紅蘿蔔的總酚含量及抗氧化活性間存在正相關性。因此，根據試驗結果，採用適當的蒸煮方法有助於提升或保持甘藷葉與紅蘿蔔的總酚含量及抗氧化活性。有關影響甘藷葉與胡蘿蔔抗氧化活性的個別生物活性成分，則有待進一步探討。

關鍵詞：甘藷葉、紅蘿蔔、總酚含量、抗氧化活性。

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